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

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Methods: Two test sample panels were sent to each laboratory which performed the analysis with their in-house assays. Recipients were blinded to sample content. The Histoplasma panel included 24 samples representing a range of Histoplasma DNA (n = 7), as well as a negative control and DNA from other fungi to test specificity (Paracoccidioides brasiliensis n = 1, Blastomyces dermatitidis n = 1, Aspergillus fumigatus n = 1, Encephalysys spp. n = 2, and Candida albicans n = 1). The Coccioides panel included 10 samples representing a range of DNA concentrations of Coccioides posadasii (n = 8), as well as a negative control and DNA from other fungi to test specificity (Uncinocarpus furfur n = 1, Pseudophytophthora occidentalis n = 1, and Candida albicans n = 1). Regarding techniques used, four laboratories used Histoplasma qPCR, and one laboratory a conventional PCR, and a broad-range PCR (uPCR) for fungal DNA. Four laboratories used different Coccioides qPCRs and one laboratory a hPCR to detect Coccioides DNA.

Results: Concerning the Histoplasma panel, qPCR assays were the most sensitive and agreement in the lowest detected amount of Histoplasma DNA was very satisfactory, ranging from 1 pg to 4 pg (<1 genomic equivalent (mean sensitivity 96.4%)). The lowest detected amount of Histoplasma DNA by qPCR (sensitivity 71.4%) and the hPCR (sensitivity 82.9%) was 0.1 and 10 pg, respectively. Overall, sensitivity ranged from 42.9-100% (mean 83.5%). Overall specificity ranged from 76.6%-100%, with false positive results occurring with high DNA concentrations (200 pg/mL) of Blastomyces spp. in two laboratories that used qPCR. Concerning the Coccioides panel, sensitivity ranged from 53.3-100% (mean 76.6%), and agreement of the lowest detected amount of Coccioides DNA by qPCR ranged from 1-16 pg (<1 genomic equivalent) (mean sensitivity, 87.5%) and in the hPCR 10 pg (sensitivity 33.3%). Specificity was between 87.5-100%, with one false positive result occurring with high DNA concentrations (20 pg/mL) of Uncinocarpus in one laboratory using qPCR.

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

The Aspergillus lateral flow assay for the diagnosis of chronic pulmonary aspergillosis

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Method: In total, 152 patient specimens were included and 42 patients were diagnosed with CPA. The pathogen was identified from sputum, BALF culture, lung resection surgery, bronchus copy biopsy, percutaneous lung biopsy and BALF GM assay.

Results: The sensitivity, specificity, PLR, NLR and kappa index of the IgG antibody test was 85.6%, 94.4%, 14.0%, 0.23% and 0.72, respectively.

Conclusions: The current work indicates that the Dynamic QuicTM Aspergillus specific IgG antibody (LFA) test shows a promising application in the diagnosis of CPA. The results are accurate and reliable, and it could be used as an aid for the early rapid screening test of CPA.

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