Total no. of samples	Galactomannan test result		KOH result	Culture
	Positive	Negative		
Serum (107)	21	86	-	-
Bronchial wash (74)	63	11	9	4
BAL (4)	4	-	3	2
Tracheal aspirate (3)	2	1	1	-

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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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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 assays. Recipients were blinded to sample content. The Histoplasma-panel included 14 samples representing a range of concentrations of Histoplasma DNA ($n = 7$), as well as a negative control and DNA from other fungi to test for specificity (*Paracoccidioides lutzii* $n = 1$; *Blastomyces dermatitidis* $n = 1$; *Aspergillus fumigatus* $n = 1$; *Emergomyces* spp. $n = 2$, and *Candida albicans* $n = 1$). The *Coccidioides*-panel included 10 samples representing a range of DNA concentrations of *Coccidioides posadasii* (n = 6), as well as a negative control and DNA from other fungi to test specificity (*Uncinocarpus reesii* n = 1; *Trichophyton violaceum* $n = 1$; and *Candida albicans* $n = 1$). Regarding techniques used, four laboratories used Histoplasma qPCRs, and one laboratory a conventional PCR and a broad-range PCR (brPCR) for fungal DNA. Four laboratories used different Coccidioides qPCRs and one laboratory a brPCR to detect Coccidioides DNA.

Results: Concerning the Histoplasma panel, qPCR assays were the most sensitives and agreement in the lowest detected amount of Histoplasma DNA was very suitable, ranging from 1 pg to 4 pg [<1 genomic equivalent (mean sensitivity: 96.4%)]. The lowest detected amount of Histoplasma DNA by cPCR (sensitivity 71.4%) and the brPCR (sensitivity 42.9%) was 0.1 and 10 pg, respectively. Overall, sensitivity ranged from 42.9-100% (mean 83.3%). Overall specificity ranged from 78.6%-100%, with false positive results occurring with high DNA concentrations (200 pg/μl) of Blastomyces spp. in two laboratories that used qPCR, Emergomyces spp. by qPCR in one laboratory and *Aspergillus* in one laboratory that used cPCR. Concerning the Coccidioides panel, sensitivity ranged from 33.3-100% (mean 76.6%), and agreement of the lowest detected amount of Coccidioides DNA by qPCR ranged from 1-16 pg (<1 genomic equivalent) (mean sensitivity: 87.5%) and in the brPCR 10 pg (sensitivity 33.3%). Specificity was between 87.5%-100%, with one false positive result occurring with high DNA concentrations (20 pg/μ) of Uncinocarpus in one laboratory using qPCR.

Conclusion: Specific protocols based on qPCR showed better sensitivity than conventional and brPCR. These methods are useful for the rapid and sensitive detection Histoplasma and Coccidioides. 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.

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

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Objectives: Chronic pulmonary aspergillosis (CPA) is an uncommon and problematic pulmonary disease, complicating many other respiratory disorders. Measurement of *Aspergillus*-specific IgG antibodies had a higher sensitivity than either IgM, IgA, or IgE and it should therefore be considered the most appropriate test for screening CPA. *Aspergillus*-specific IgG antibody levels have been successfully used to monitor the response of CPA to medical therapy. Recently, a novel rapid test for *Aspergillus*-specific IgG antibody from Dynamiker Biotechnology (Tianjin) Co., Ltd. was released as a screening test of CPA. It is a fluorescent immunochromatographic cassette test using a monoclonal antibody against *Aspergillus*-specific IgG antibody and Europium nanoparticles (Eu NP) (Fig. 1). It is a semi-quantitative test which was easy to operate, rapid with portable detection devices, and can be widely accepted by clinical and primary medical (Fig. 2). We collect clinical samples to verify the detection performance of the Dynamiker Quic™ Aspergillus-specific IgG antibody (LFA) test.

Methods: In total, 102 patients were included and 42 patients were diagnosed with CPA. The pathogen was identified from sputum, BALF culture, lung resection surgery, bronchos copy biopsy, percutaneous lung biopsy and BALF GM assay.

Results: The sensitivity, specificity, PLR, NLP and Kappa index of the IgG antibody test were 85.6%,94.4%,14.0%,0.23% and 0.72, respectively.

Conclusions: The current work indicates that the Dynamiker Quic™ 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.

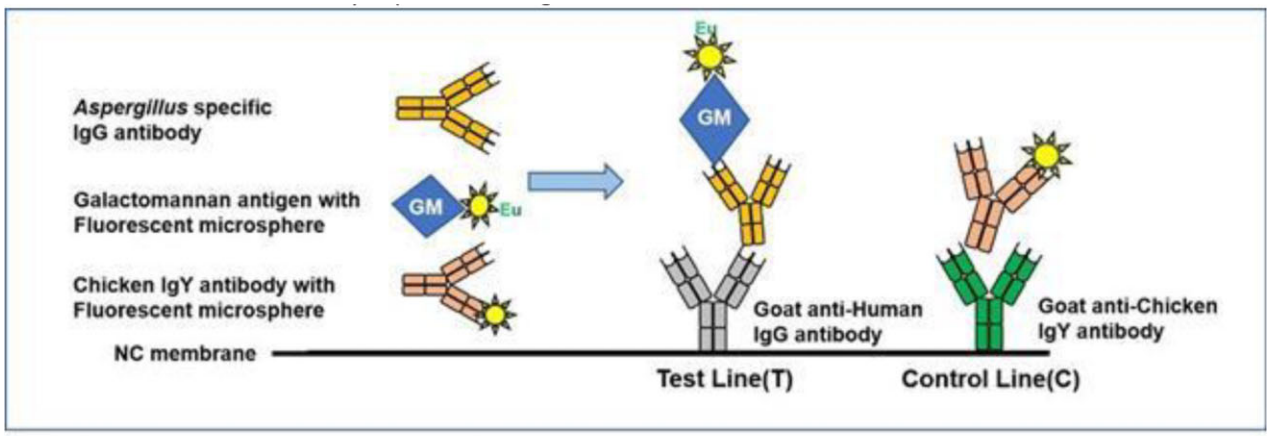

Figure 1. Diagram of the Dynamiker *Aspergillus* specific IgG antibody (LFA)