Evaluation of a new lateral flow device for the detection of anti-Aspergillus antibodies and comparison to the Western Blot

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Introduction

Molds from the genus *Aspergillus* are ubiquitous. Some of them, mainly *A. fumigatus*, are opportunistic pathogens. They can affect either immunocompetent and immunosuppressed host with a wide range of diseases, some of them lethal without treatment, and are affecting millions of people over the world. Diagnosis of those disease challenging and relies on multiple criteria. For the immunocompetent host, proof of exposure to *Aspergillus* with serology, mainly IgG response, is a major criteria of the diagnosis. Many serological techniques are available, however they are not suited for resource-poor settings, being too expansive and/or relying on a stable electric supply. We present here the evaluation of a lateral flow immunochromatographic (ICT) technique for the detection of anti-*Aspergillus* antibodies in the serum or plasma. This technique produces result in less than 30 minutes with minimal laboratory equipment needed.

Materials and methods

- The evaluation was composed of two studies :
- ❖ A 4 month single center prospective study (Marseille, France): 44 cases and 210 controls.
 - ❖ A multicentric retrospective study (Bordeaux, Marseille, Montpellier, Rennes and Toulouse, France): 262 cases and 188 controls

- Case definition was based on consensus conference:
- ❖ Allergic BronchoPulmonary Aspergillosis (ABPA)¹
 - ❖ Chronic Pulmonary Aspergillosis (CPA)²
 - ❖ Chronic colonization in CF³
 - ❖ Acute and Sub-Acute Invasive Aspergillosis (IA-SAIA)⁴
 - ❖ All other proven localized aspergillosis (abcesses, sinusitis, ...)

Controls definition: patients who underwent aspergillus serology testing but did not matched with the above definitions.

Serological techniques used to detect anti-*Aspergillus* antibodies were as follow : Elisa Biorad® (Rennes, Bordeaux, Montpellier), WB *Aspergillus* LDBIO (Montpellier, Bordeaux), Elisa Serion® (Toulouse, Marseille), IEP (Toulouse, Marseille, Rennes).

Aspergillus ICT was used by dispensing 15µL of serum or plasma sample then 4 drops of the eluting solution. Results were read with naked eye 20 to 30 minutes later. Positivity of the test was give by the presence of a grey or black line under the “T” sign (see fig).

For all centers where WB was not done as part of the routine follow-up of patients it has been made retrospectively expect for 7 samples from the prospective study (insufficient volume, 2 cases and 5 controls). WB was used according to manufacturer’s instruction. Example of positive WB is shown (see fig).

For both test, Sensibility (Se), Specificity (Sp) and their 95% confidence intervals (95CI – Wilson’s method with correction of continuity) were calculated using clinical status as gold standard. Agreement between both test was given using Cohen’s kappa.

Fig left: Positive (top) and negative (bottom) ICT

Fig right: Example of positive WB: 2 out of the 4 bands shown are needed

Conflict of interest	
RP received a PhD grant and SR a travel grant from LDBIO Diagnostics. others: none to declare.	

- Prospective study:

ICT was positive for 40/44 cases and negative for 211/219 controls Se = 90.9% Sp = 96.3%	WB was positive for 36/42 cases and negative for 192/214 controls. Se = 85.7% Sp = 89.7%
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- Retrospective study:

ICT was positive for 232/262 cases and negative for 181/188 controls. Se = 88.5% Sp = 96.3%	WB was positive for 247/262 cases and negative for 187/188 controls. Se= 94.3% Sp = 99.5%
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- Total : (disease per disease results in table 1)

ICT was positive for 272/306 cases and negative for 392/407 controls. Se = 88.9% Sp = 96.3%	WB was positive for 283/304 cases and negative for 379/402 controls. Se = 93.1% Sp = 94.3%
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Table 1 : disease per disease results for ICT and WB

Aspergillus disease	ICT	WB
ABPA	68/73 (93%)	72/73 (99%)
APC	72/78 (92%)	77/78 (99%)
Colonization	84/94 (89%)	81/92 (88%)
IA-SAIA	16/24 (67%)	19/24 (79%)
Others	32/37 (86%)	34/37 (92%)
Total positive (Se) [95CI]	272/306 (89%) [84,7-92,1%]	283/304 (93%) [89,5-95,6%]
Controls (Sp) [95CI]	392/407 (96%) [93,8-97,9%]	379/402 (94%) [91,3-96,3%]

- ICT/WB comparison
Agreement between both technique was considered to be very good : 657/706 samples gave the same results (93%).
Cohen's kappa: 0,858 [0,819-0,896].

Conclusion

The new *Aspergillus* ICT kit showed excellent performances with 89% sensitivity and 96% specificity. Those performances were very closed of those of the WB (93% sensitivity, 94% specificity). Agreement between both techniques was very good (Kappa 0,858). Due to its ease of use, the ICT brings new diagnostic opportunities in resource-poor settings.

References

1 Agarwal R., et al. 2013. « Allergic Bronchopulmonary Aspergillosis: Review of Literature and Proposal of New Diagnostic and Classification Criteria ». Clinical and Experimental Allergy 43 (8):850-73.
2 Denning D, et al. 2016. « Chronic Pulmonary Aspergillosis: Rationale and Clinical Guidelines for Diagnosis and Management ». The European Respiratory Journal 47 (1):45-68.
3 Oliva A, et al. 2015. « Evaluation of the Aspergillus Western Blot IgG Kit for Diagnosis of Chronic Aspergillosis ». Journal of Clinical Microbiology 53 (1):248-54.
4 De Pauw, B, et al. 2008. « Revised Definitions of Invasive Fungal Disease from the EORTC/ MSG Consensus Group ». Clinical Infectious Diseases 46 (12):1813-21.