50° CONGRESSO NAZIONALE AMCLI
PALACONGRESSI DI RIMINI
24 - 27 MARZO 2023

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA
Azienda Ospedaliero - Universitaria di Bologna
Policlinico S. Orsola-Malpighi

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COMPARISON OF ASPERGILLUS GALACTOMANNAN ANTIGEN DETECTION IN RESPIRATORY AND SERUM SAMPLES USING TWO DIFFERENT IMMUNOENZYMATIC ASSAYS

A. Primavera[1], G. Roncarati[2], G. Lombardi[2], M. Giannella[3], T. Lazzarotto[1]

1. U.O. Microbiologia, DIMEC, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Università di Bologna, Bologna
2. U.O. Microbiologia, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna
3. U.O. Malattie Infettive, DIMEC, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna

INTRODUCTION

Invasive forms of Aspergillus infection remain difficult to diagnose and to treat. The detection of Aspergillus galactomannan in serum and respiratory samples (RS), in conjunction with other diagnostic procedures such as microbiological culture, histological examination of biopsy samples and radiographic evidence, can be used as an aid in the diagnosis of Invasive Aspergillosis.[1,2] Our aim was to compare two different immunoassays for the detection of galactomannan: Platelia™ Aspergillus Ag immunoassay (Biorad) and CHORUS Aspergillus Galactomannan Ag (Diesse).

METHODS

A total of 267 samples (from January 2021 to June 2022), attributable to 184 patients, was tested at the Microbiology Unit of the Policlinico di Sant'Orsola (Bologna) for routine fungal assays: 182 were RS (34 bronchoalveolar lavages and 148 bronchial aspirates) while 85 were serum samples. 126 samples from males and 58 from females (mean age 61.7 ± 17,55 , range 2-95 year), were included in our study (Fig.1A). The main hospital units from which the tests were required are shown in Figure 1B: 50% of patients came from Intensive Care Unit or Hematology/Oncology units. Each sample was first tested using Platelia assay (Fig.2B) currently used in our routine, with a cut-off Index of 0.5 according to manufacturer's instructions, then the same samples were tested with CHORUS (Fig.2A) assay (cut-off Index = 0.9). In both cases samples were pre-treated with a buffer containing EDTA and heated in order to dissociate immune complexes and precipitate proteins that could interfere with the test. The flow charts in Figure 3A and 3B represent the working procedures of the two kits.

The results obtained from the two methods were analysed through Cohen's kappa coefficient. We defined agreement and disagreement rates between the two tests by calculating the Overall Percent Agreement (OPA), the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA), for the total of samples first, than for the respiratory samples group and for serum samples group. Then we calculated the Positive Predictive Value (PPV) and the Negative Predictive Value (NPV). For a small number of sample (67) it was possible to compare the Galactomannan result with a clinical reference pattern consisting of establishing the presence of clinical criteria of proven or probable Invasive Aspergillosis.

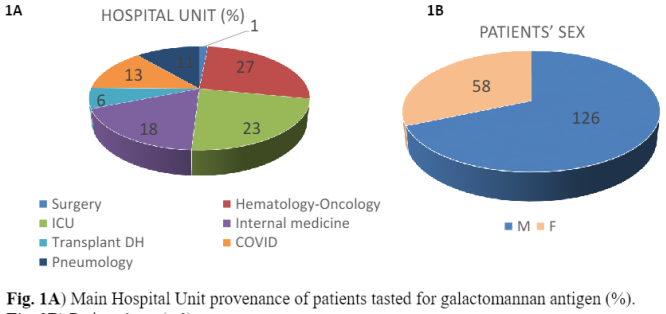

Fig. 1A) Main Hospital Unit provenance of patients tasted for galactomannan antigen (%).
Fig. 2B) Patients' sex (n.°)

Fig 2A) CHORUS TRIO instrument with autosampler
Fig 2B) PLATELIA™ Aspergillus Ag test.

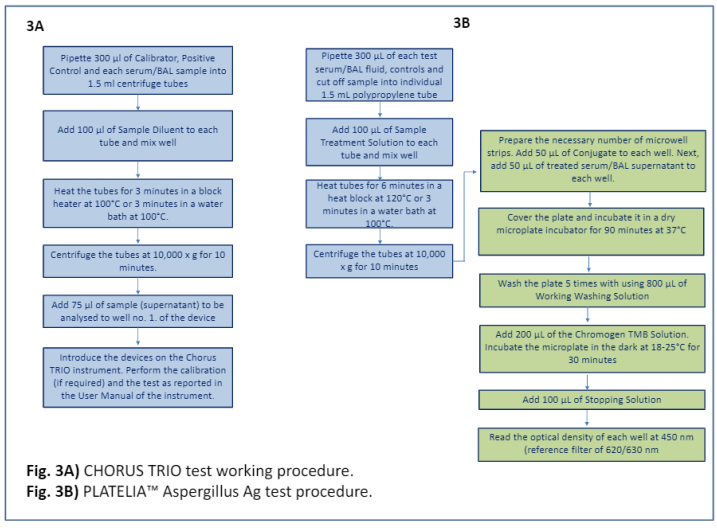

Fig. 3A) CHORUS TRIO test working procedure.
Fig. 3B) PLATELIA™ Aspergillus Ag test procedure.

RESULTS

The two immunoassays revealed an OPA of 76,4%, a PPA of 68.7% and a NPA of 82.6%. Specifically, RS and serum samples showed an OPA of 68.1% and 94.1%, a PPA of 68.9% and 66.7% and a NPA of 67.1% and 98.6% respectively The overall Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were 75.7% and 76.9% for RS + serum samples; for only RS the PPV was 74.5% and the NPV was 60.7%; for serum samples the PPV was 98.6% and 94.7% (Fig. 4A).

5/85 serum specimens demonstrated discordant results between the two tests: 4/5 result Platelia-positive and CHORUS-negative, while 1/5 was CHORUS-positive and Platelia-negative.

33/182 RS resulted Platelia-positive and CHORUS-negative, while 25/182 resulted CHORUS-positive and Platelia-negative.

Clinical data were available for 45 samples (discordant group) and 22 showed clinical symptoms consistent with Aspergillosis: 13 resulted Platelia-positive (59%) while 9 CHORUS-positive (41%).

199/267 samples (75%) performed a microbial culture on RS: Aspergillus spp. grew in 21/199 cultures. 6/21 had a serum galactomannan result (3 positive for both immunoassays, 2 negative for both immunoassays; 1 Platelia-positive and CHORUS-negative). 15/21 had a respiratory galactomannan result (13 positive for both tests, 1 negative for both tests; 1 CHORUS-positive and Platelia-negative). The detection of other fungi and bacteria in our samples (mainly Candida Albicans, Pseudomonas Aeruginosa, Klebsiella Pneumoniae) results equally distributed in both tests, not justifying the anomalies of discordant samples.

4A

% AGREEMENT	OPA	PPA	NPA	PPV	NPV	Cohen's k
RS+SERUM SAMPLES	76.4%	68.7%	82.6%	75.7%	76.9%	0.5
RS	68.1%	68.9%	67.1%	74.5%	60.7%	0.7
SERUM	94.1%	66.7%	98.6%	88.9%	94.7%	0.4

Fig. 4A) In this table the OPA, PPA, NPA, PPV, NPV, and Cohen's kappa coefficient calculated for the total of samples, for respiratory samles and for serum samples are reported .

CONCLUSIONS

Overall the two immunoassays showed comparable performance. The CHORUS test has been proved to be a useful alternative for diagnosis due to its user friendly format. The Platelia test requires samples accumulation and batching while the Chorus test facilitates its use with a single sample. Therefore, the Chorus test can replaces the Platelia one when a quicker and single response is necessary. However, a pre-treatment step is necessary for both tests that could introduce a possible sample contamination. In both tests false positive results can occur in patients exposed to products that may have been contaminated by fungal remains.[3] Moreover some patients with demonstrated bacterial disease could have a positive galactomannan result.[4] Further clinical data could improve results interpretation and performance evaluation of the two tests.

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