ORIGINAL ARTICLE

Performance of the IMMY® and OLM® lateral flow assays for *Aspergillus* galactomannan compared to fungal culture and Platelia™ galactomannan enzyme immuno-assay

Wendy P McKinney, Bronwyn Findon, Chris Mansell, Sally A Roberts and Arthur J Morris

ABSTRACT

Objectives: To investigate the performance of two lateral flow assays (LFAs) detecting *Aspergillus* antigens, galactomannan (GM) or glycoprotein antigen, compared with *Aspergillus* culture and GM enzyme immunoassay (EIA) results.

Methods: Lower respiratory tract samples, either bronchoalveolar lavage or bronchial wash specimens, were tested with the Platelia™ GM EIA, IMMY® LFA, or OLM® LFA. Test results were compared to culture and for both LFAs compared to the GM EIA. Three cut-off indexes were used for the GM EIA, ≥0.5, ≥1.0 and ≥1.5. Two optical density (OD) cut-offs were used for the IMMY LFA ≥0.5 and ≥1. OD readers were used to interpret both LFAs. Positive and negative predictive values (PPV, NPV) were calculated for clinically encountered prevalence rates of 5%, 10% and 15%.

Results: The sensitivity of the GM EIA for a positive *Aspergillus* culture was unaltered by the OD value used to define a positive. For *Aspergillus* culture the specificity, PPVs, test concordance and diagnostic odds ratio all increased with increasing GM EIA index cut-off values from ≥0.5 to ≥1.5. The OLM LFA and the IMMY LFA with the 1.0 OD cut-off had similar performance values for positive *Aspergillus* culture results. Both LFA assays had NPVs of 89%-97% vs. 94%-98% for GM EIA. Most specimens with either a positive GM EIA or LFA and a negative *Aspergillus* culture result were either culture negative, 29-60%, or for GM EIA and OLM LFA grew yeasts, 22 -73%, not known to have cross-reacting antigens.

Conclusions: Both LFAs are user friendly. The test performance of the IMMY LFA is improved when an OD of ≥1 is used to define a positive and at this cut-off the results of both LFAs are comparable. Positive assays with negative *Aspergillus* cultures are not due to the presence of cross-reacting fungi. Both LFAs had high NPVs which help rule out invasive aspergillosis.

Keywords: Aspergillosis, *Aspergillus,* lateral flow assay, enzyme-immunoassay, fungal diagnosis, point-of-care assays.

N Z J Med Lab Sci 2022; 76(1): 27-31

INTRODUCTION

Invasive aspergillosis (IA) is a significant fungal infection in immunocompromised patients with considerable morbidity, mortality (~30%) and associated cost (1-3). Diagnosis of IA is challenging because obtaining tissue from the site of putative infection for direct examination, culture and histopathology is often difficult and at times impossible. This diagnostic difficulty has driven the use of biomarker assays for *Aspergillus* antigens in serum or bronchoalveolar fluid (BAL) to assist in the diagnosis of IA (4-7).

The most widely used, and reviewed, biomarker assay is the Platelia™ sandwich enzyme-linked immunosorbent assay (EIA), (Bio-Rad, Marnes-La-Coquette, France), which detects galactomannan (GM) released from growing *Aspergillus* hyphae *in vivo* (8). While relatively specific for *Aspergillus* GM other fungi, e.g., *Penicillium*, *Fusarium* and *Paecilomyces* spp., can cross-react and contaminate GM in antibiotics and GM present in foodstuffs may cause false positive results (9,10). When used on BAL specimens from adult patients meeting published definitions for proven or probable IA (11), the GM EIA has sensitivity and specificity values of 78-90% and 81-94% depending on the cut-off values used (4,5,8,12). For BALs from paediatric patients the corresponding proportions were 82% and 88% respectively (7). While the Platelia™ EIA can be run as a "one-off" test, low test volumes mean many laboratories perform batch testing which increases result turnaround time and potentially delays diagnosis.

Immuno-chromatographic lateral flow assays (LFAs) have been developed to detect *Aspergillus* antigens in serum and BAL specimens. Their format is attractive as they have the potential of being a rapid method to detect an *Aspergillus* biomarker to assist in the diagnosis, or exclusion, of IA. The IMMY LFA (sōna *Aspergillus* Galactomannan LFA, Norman, OK, USA) uses a propriety mixture of two monoclonal antibodies to detect GM. Cross-reactivity with *Candida* spp. and non-*Aspergillus* moulds has been reported (13,14). In small studies, with specimens from neutropenic and non-neutropenic patients, the sensitivity and specificity ranges from 58-89% and 68-88% respectively (15,16). The OLM LFA (OLM *Aspergillus* LFD [*Asp*LFD], OLM Diagnostics, Newcastle Upon Tyne, England), uses a monoclonal antibody which binds to an extracellular glycoprotein antigen secreted constitutively during the active growth of *Aspergillus* spp (9). The assay detects the target antigen in a broad range of *Aspergillus* spp. as well as many *Paecilomyces* and *Penicillium* spp. but does not react with antigens from a wide range of fungi including *Candida albicans*, *Cryptococcus neoformans*, *Fusarium* and *Rhizopus* spp. (9). For BAL specimens, from patients with proven or probable IA, the assay has a sensitivity of 86% and specificity of 93% (17). When used on BALs, from a range of patient groups at risk for pulmonary IA, this LFA had higher specificity than the GM EIA and was considered a rapid and simple test to rule out IA (18).

The aim of this study was to compare the results of the GM EIA and both LFAs to fungal culture results, not only the recovery of *Aspergillus* isolates but also species known to cross -react in biomarker assays. The LFA results were also compared to GM EIA results.

MATERIALS AND METHODS

Only lower respiratory samples, either bronchoalveolar lavage or bronchial wash (BAL/BW) specimens, sent for either fungal culture or GM testing were used in this study. Testing for GM using the Platelia™ immunoenzymatic sandwich microplate assay (Platelia™ *Aspergillus* Ag Kit, Bio-Rad, Marnes-la-Coquette, France) was performed at Waikato Hospital. BAL/BW specimens were received from referral laboratories as an aliquot of the original sample. They were transported in a bio-freeze bottle and sent by same-day courier. If testing is not performed on the day of arrival, samples were stored at -20°C for a maximum period of three days before testing. Following testing, samples were stored at -20°C before being sent in bio-freeze bottles by same-day courier to Auckland Hospital for LFA testing.

GM EIA testing followed the manufacturer's instructions. Briefly, 300µL of sample was added to 100µL of sample treatment solution, vortexed, heated at 120°C in a heat block for six minutes, then centrifuged at 10,000 x g for 10 minutes. Next, 50µL of the supernatant and 50µL of conjugate were incubated in antibody pre-coated microplates for 90 minutes at 37°C. The plates were washed five times and then incubated with 200µL of substrate chromogen reaction solution for 30 minutes, in the dark at room temperature. The reaction was stopped with 1N sulphuric acid, and the plates were read at an optical density (OD) of 450nm, with a reference filter of 630nm. Positive, negative, and cut-off controls were included in each batch. The presence or absence of GM antigen in each patient specimen was determined by calculation of an index, this being the OD value of the specimen divided by the mean OD of the wells containing cut-off control serum.

While the positive GM EIA cut-off for BAL specimen is an index of ≥0.5 the manufacturer states that indexes between 0.5-1.0 have lower predictive values than specimens with results of >1.0 and that indexes between 0.5-1.0 should be interpreted in light of other clinical, radiological or laboratory evidence of IA (10). Local experience, comparing GM EIA indexes to clinical cases meeting published definitions of IA (11), has led to this cautionary range being 0.5-<1.5 with specimens having an index of ≥1.5 being reported as "Reactive. Evidence for invasive aspergillosis". To allow comparison with publications using different cut-off values for positive GM EIA results, performance was calculated for indexes of ≥0.5, ≥1.0 and ≥1.5 (8,12,19).

All LFA testing was performed at Auckland Hospital using the IMMY LFA (sōna Aspergillus Galactomannan LFA, Norman, OK, USA) and the OLM LFA (OLM Aspergillus Lateral Flow Device [*Asp*LFD], OLM Diagnostics, Newcastle Upon Tyne, England). Samples had been collected over a nine-month period, stored at -20°C then thawed at room temperature before testing. Testing was performed in accordance with manufacturer's instructions for both assays. Briefly, for the IMMY LFA 300µL of BAL/BW was added to a microcentrifuge tube and 100µL of pre-treatment buffer added. The vortexed sample was placed into a heat block at 120°C for 6-8 minutes, then spun at 14,000 x g for 5 minutes, and 80µL of supernatant added to a microwell tube containing 40µL of running buffer, the test strip was added and run for 30 minutes. For the OLM LFA haemorrhagic samples were centrifuged for 1 minute, 150µL sample added to 300µL of sample buffer and heated for 3 minutes at 120°C, then centrifuged at 14,000 x g for 5 minutes, then 70µL of supernatant added to the LFA cassette port and run for 30 minutes. For non-haemorrhagic samples were spun at 14,000 x g for 1 minute and 70µL of supernatant was added to the sample port and run for 15 minutes.

Results were read and interpreted using the optical density digital cube reader supplied by each manufacturer. The manufacturer's recommended optical density (OD) using the IMMY LFA cube reader for a positive is ≥0.5. No numeric value is displayed with the OLM Cube Reader; test results are displayed as POS in the event of a positive *Aspergillus* result, VLP in the event of a very low positive result, NEG in the event of an *Aspergillus* negative result, or FAIL. Both POS and VLP were recorded as positive results for analysis.

Positive and negative predictive values (PPV, NPV) were calculated by assuming prevalence rates for invasive aspergillosis (IA) of 5% (a common rate for patients undergoing remission-induction therapy with antifungal prophylaxis) (3), 10% (observed after allogeneic stem cell transplant with antifungal prophylaxis and recently reported rate in a high-risk Australian cohort) (6,20), and 15% to represent a higher prevalence group observed in a recent Australian study reporting proven and probable IA in high risk haematology patients (20).

Statistical analysis was performed using VassarStats, (website; www.VassarStats.net). The Fischer Exact two-tail test was applied to test differences between assays. If the sample size was too large, the Chi-square test was applied. Significance was defined as a P value <0.05.

Ethical approval was not required by New Zealand Health and Disability Standards for this type of laboratory assay evaluation.

RESULTS

One hundred and seventy-nine samples were tested from 132 patients: 23 patients with two samples, six with three and one each with five or nine samples. One sample, a thick mucoid specimen, gave invalid test results with both LFAs and was excluded from analysis. Another sample gave an invalid OLM LFA results but was positive by the IMMY LFA (OD 2.2). This sample is excluded from OLM LFA test performance analysis. Neither of these samples underwent GM EIA testing. Patient samples were received from clinical services looking after patients at risk for IA, e.g., lung transplant recipients or those with haematological malignancies, clinical histories were not collected.

Assay performance against fungal culture results is shown in Table 1. The sensitivity of the GM EIA was unaltered by the index value used to define a positive. The specificity and test concordance did not increase with increasing index cut-off values from 0.5 to 1.5 (P values ≥0.18). Positive predictive values were higher at all prevalence rates with GM EIA indexes ≥1.5 than ≥0.5 (P<0.01 all comparisons) but not statistically significant for GM EIA indexes of ≥1 (P values ≥0.14).

Most GM EIA positives with negative *Aspergillus* culture had either no fungal isolate recovered or had yeasts present, 80-91%. Only one specimen grew a *Penicillium* sp., footnotes Table 1. For the IMMY LFA an OD cut-off value of 1.0 had lower sensitivity (P=0.02), higher specificity (P<0.001), PPVs (P<0.02 all prevalence rates) and concordance (P<0.001) than a 0.5 cut-off, Table 1. The OLM LFA and the IMMY LFA with the 1.0 OD cut-off had similar performance values for sensitivity (P=0.8), specificity (P=0.12), PPVs (P>0.13 for all prevalence rates) and concordance (P=0.35), Table 1. Most positive LFA assays which did not have *Aspergillus* cultured had either negative fungal cultures, 50-60%, or for OLM LFA had yeasts recovered which are not known to cross react, 22%, footnotes Table 1. A minority, 17-22%, grew a non-speciated *Penicillium* isolate. Both LFA assays had NPVs of 89-97% vs. 94-98% for GM EIA. Both LFAs had very similar NPVs at all prevalence rates (P values 0.89-0.92).

The performance of the LFAs compared with the GM EIA, at cut-off values of ≥0.5, ≥1.0 and ≥1.5, are shown in Table 2. For the IMMY LFA specificity, at each GM EIA cut-off, was significantly higher with the ≥1 OD cut-off vs. the ≥0.5 OD cut-off, P<0.001 at each GM EIA cut-off. The IMMY LFA with ≥1 OD cut-off and OLM LFA had similar performance characteristics at each GM EIA cut-off with non-statistically different sensitivity (P values 0.99-1.0), specificity (P values 0.17-0.23), or concordance (P values 0.09-0.27), Table 2.

There were 56 samples tested by all three assays. Using the 1.0 OD cut-off for the IMMY LFA, both LFAs had no differences in their sensitivity, specificity, PPV or test concordance compared with GM EIA results at ≥0.5, ≥1.0 or ≥1.5 cut-off values (data not shown). Importantly the NPVs for the IMMY LFA (84, 89%, 95%) and the OLM LFA (86%, 93%, 98%), at the three respective GM EIA cut-offs, were not statistically different, P values all >0.6.

The time taken for a one-off GM EIA would be approximately 2.5 hours, for IMMY LFA approximately 45 minutes and for the OLM LFA haemorrhagic and non-haemorrhagic specimens approximately 40 and 20 minutes respectively. Fifty-seven specimens (32%) in this series were haemorrhagic.

All data was under the control of the authors and while both LFA manufacturers were provided a preliminary draft to comment on the content and decision to publish were the authors decisions.

Table 1. Galactomannan assay results compared to *Aspergillus* culture results.

		***Aspergillus* culture (%)**		**Test performance (%)**				**Positive predictive value (%)/ Negative predictive value (%) for prevalence of**		
Assay	**Result**	**Positive**	**Negative**	**Sensitivity**	**Specificity**	**Concordance**	**Diagnostic Odds Ratio**	**5%**	**10%**	**15%**
Galactomannan EIA, ≥0.5 positive (n=69)	Positive	5 (31)	11* (69)	71	82	81	11.6	17/98	30/96	41/94
	Negative	2 (4)	51 (96)							
Galactomannan EIA, ≥1.0 positive (n=69)	Positive	5 (42)	7† (58)	71	89	87	19.6	26/98	42/97	54/95
	Negative	2 (4)	55 (96)							
Galactomannan EIA, ≥1.5 positive (n=69)	Positive	5 (50)	5‡ 50)	71	92	90	28.5	32/98	50/97	61/95
	Negative	2 (3)	57 (97)							
IMMY LFA ≥0.5 positive (n=156)	Positive	24 (26)	70§ (74)	75	44	50	2.3	7/97	15/94	19/91
	Negative	8 (13)	54 (87)							
IMMY LFA ≥1.0 positive (n=156)	Positive	14 (41)	20** (59)	44	84	76	4.0	13/95	23/93	33/89
	Negative	18 (15)	104 (85)							
OLM LFA (n=162)	Positive	15 (32)	32†† (68)	48	76	70	2.9	10/97	18/93	26/89
	Negative	16 (14)	99 (86)							

* No growth (n=5), *Candida albicans* (n=2), yeast-unidentified (n=3), *Penicillium* sp. (n=1). † No growth (n=2), *C. albicans* (n=1), yeast-unidentified (n=3), *Penicillium* sp. (n=1).
‡ No growth (n=2), *C. albicans* (n=1), yeast-unidentified (n=1), *Penicillium* sp. (n=1). § No growth (n=40), *C. albicans* (n=9), yeast-unidentified (n=6), *Penicillium* spp. (n=12), other moulds (n=3); *Exophiala dermatitidis*, *Rhizomucor miehei* and an unidentified mould. ** No growth (n=12), *C. albicans* (n=1), yeast-unidentified (n=2), *Penicillium* spp. (n=4), other mould (n=1); *R. miehei.* †† No growth (n=16), *C. albicans* (n=5), yeast-unidentified (n=2), *Penicillium* spp. (n=7), other moulds (n=2); *Paecilomyces variotii*, *R. miehei*.

Table 2. Lateral flow assay results compared to Galactomannan enzyme-immunoassay (GM-EIA) results

		GM-EIA		**Test performance (%)**		
Assay	**Result**	**Positive ≥0.5 (%)**	**Negative (%)**	**Sensitivity**	**Specificity**	**Concordance**
IMMY LFA ≥0.5 positive (n=67)	Positive	15 (34)	29 (66)	94	43	55
	Negative	1 (4)	22 (96)			
IMMY LFA ≥1.0 positive (n=67)	Positive	10 (67)	5 (33)	63	90	84
	Negative	6 (12)	46 (88)			
OLM LFA (n=56)	Positive	10 (56)	8 (45)	63	80	75
	Negative	6 (16)	32 (84)			
		Positive ≥1.0	**Negative**	**Sensitivity**	**Specificity**	**Concordance**
IMMY LFA ≥0.5 positive (n=67)	Positive	12 (27)	32 (73)	100	42	52
	Negative	0 (0)	23 (100)			
IMMY LFA ≥1.0 positive (n=67)	Positive	9 (60)	6 (40)	75	89	87
	Negative	3 (6)	49 (94)			
OLM LFA (n=56)	Positive	8 (44)	10 (56)	67	77	75
	Negative	4 (11)	34 (89)			
		Positive ≥1.5	**Negative**	**Sensitivity**	**Specificity**	**Concordance**
IMMY LFA ≥0.5 positive (n=67)	Positive	10 (23)	34 (77)	100	40	49
	Negative	0 (0)	23 (100)			
IMMY LFA ≥1.0 positive (n=67)	Positive	9 (60)	6 (40)	90	89	90
	Negative	1 (2)	51 (98)			
OLM LFA (n=56)	Positive	8 (44)	10 (56)	80	78	79
	Negative	2 (6)	36 (94)			

DISCUSSION

In this LFA assay evaluation only rare samples gave invalid results. The manufacturer-provided OD cube readers were straightforward to use. LFA provides an option for rapid "one off" biomarker testing.

The cut-off used to define a positive GM EIA determines test performance (5,8,11,18). We did not observe altered sensitivity at different criteria, index values 0.5-1.5, but did show higher specificity with higher cut of values, as observed by others (8,12,19).

It has been noted that GM EIA and LFAs for *Aspergillus* may cross react with other fungi, e.g., GM EIA with *Penicillium*, *Paecilomyces*, and *Alternaria* spp. (10). In a study of 60 BAL specimens Pasqualotto et al found no association between lung colonization with either *Penicillium* or *Candida* species (21). In this study most assay "false positives" for *Aspergillus* culture-negative samples were not due to the presence of known cross-reacting organisms. We believe ours is the first report that has systematically investigated this issue for LFAs. Both LFAs showed modest concordance with the GM EIA with lower sensitivity and comparable specificity for culture positive samples.

Estimating the performance of assays requires applying the sensitivity and specificity values to clinically relevant prevalence rates, an approach used when evaluating assays used for diagnosing IA (22). We used IA prevalence rates of 5%, 10% and 15% to reflect encountered rates of IA in immunocompromised populations, including two Australian studies (3,6,20). PPVs were low to modest, 7-≤50%, for most assays at each prevalence rate and only reached 54-61% for GM EIA 1.0 and 1.5 cut offs for 15% prevalence, respectively. NPVs however were high with the NPVs for both LFAs 93-97% when IA prevalence was 5-10%. Negative predictive values for the LFAs, fell to <90% only for the highest prevalence population. Two recent reports evaluating both LFAs against published definitions of proven and probable IA (11) observed lower NPVs, 82-91%, to ours (23,24).

We observed better performance with the IMMY LFA when applying an OD cut-off of 1.0. We have been informed by the IMMY LFA manufacturer that based on accumulating data they are planning to reword their "Information for users" along the lines of "Indexes between 0.5-1.0 have lower predictive values than specimens with results of >1.0. Indexes between 0.5-1.0 should be interpreted carefully with consideration of other clinical, radiological or laboratory evidence of IA". (Aaron Dumas, IMMY Inc., personal communication) When a 1.0 cut-off was used for the IMMY LFA both LFAs had indistinguishable test performance.

Both LFAs were straightforward to use. The IMMY LFA total test time was slightly longer (~45 minutes) than for haemorrhagic specimens requiring pre-treatment for the OLM LFA (~40 minutes), with non-haemorrhagic specimens taking ~20 minutes. Haemorrhagic specimens were not infrequent (32%).

Being an *in vitro* evaluation of assays, this study has limitations. No clinical data such as radiology, treatment, immunosuppression, or presence and degree of neutropenia, were collected. This prevents test performance being evaluated directly against published definitions for proven, probable, or possible IA (11). Not all samples were tested by all three assays, and the modest number of GM results limited GM EIA-LFA comparisons. Only BAL specimens were included preventing comment on performance with serum samples. Samples had been stored at -20°C before LFA testing and were not tested contemporaneously in same laboratory. However, GM is stable in BAL samples stored at -20°C for many years and we do not expect that the short-term storage at -20°C used here affected test results (23,25).

Conclusions: In summary both LFAs were straightforward to use and invalid results rare. A cut-off of 1.0 for the IMMY LFA improved its test performance. Both LFAs had high NPVs which support their use as a rapid one-off test to help rule out IA.

ACKNOWLEDGEMENTS

Manufacturers provided either all (IMMY) or most (OLM) LFA strips free of charge for this evaluation. Both manufacturers provided their cube readers for the evaluation period.

AUTHOR INFORMATION

Wendy P McKinney, BMLS, Medical laboratory Scientist[1]
Bronwyn Findon, BMLSc, Medical laboratory Scientist[2]
Chris Mansell, MBChB FRCPA, Clinical Microbiologist[2]
Sally A Roberts, MBChB FRACP FRCPA, Clinical Microbiologist[1]
Arthur J Morris, BSc(Hons I) MD D(ABMM) FRCPA, Clinical Microbiologist[1]

[1] New Zealand Mycology Reference Laboratory, LabPLUS, Auckland City Hospital, Park Road, Auckland, New Zealand
[2] Microbiology Laboratory, Waikato Hospital, Hamilton, New Zealand.

Correspondence: Dr Arthur J Morris. Microbiology Laboratory, LabPLUS, Auckland City Hospital, Auckland, New Zealand.
E-mail: arthurm@adhb.govt.nz

REFERENCES

1. Brown GD, Denning DW, Gow NA, et al. Hidden killers: human fungal infections. *Sci Transl Med* 2012; 4(165): 165rv13.
2. Slavin M, Fastenau J, Sukarom I, et al. Burden of hospitalization of patients with Candida and Aspergillus infections in Australia. *Int J Infect Dis* 2004; 8:111-120.
3. van de Peppel RJ, Visser LG, Dekkers OM, de Boer MGJ. The burden of invasive aspergillosis in patients with haematological malignancies: A meta-analysis and systematic review. *J Infect* 2018; 76: 550-562.
4. Maertens J, Maertens V, Theunissen, K, et al. Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematological diseases. *Clin Infect Dis* 2009; 49(11): 1688-1693.
5. Guo Y-L, Chen Y-Q, Wang K, et al. Accuracy of BAL galactomannan in diagnosing invasive aspergillosis: a bivariate meta-analysis and systematic review. *Chest* 2010; 138: 817-824.
6. Morrissey CO, Chen SC-A, Sorrell TC, et al. Galactomannan and PCR versus culture and histology for directing use of antifungal treatment for invasive aspergillosis in high-risk haematology patients: a randomised controlled trial. *Lancet Infect Dis* 2013; 13(6): 519-528.
7. Lehrnbecher T, Robinson PD, Fisher BT, et al. Galactomannan, β-D-glucan, and polymerase chain reaction-based assays for the diagnosis of invasive fungal disease in pediatric cancer and hematopoitic stem cell transplantation: a systematic review and meta-analysis. *Clin Infect Dis* 2016; 63(10): 1340-1348.
8. De Heer K, Gerritsen MG, Visser CE, Leeflang MMG. Galactomannan detection in broncho-alveolar lavage fluid for invasive aspergillosis in immumocompromised patients. *Cochrane Database Syst Rev* 2019; 5(5): CD012399.
9. Thornton CR. Development of an immunochromatographic lateral-flow device for rapid serodiagnosis of invasive aspergillosis. *Clin Vaccine Immunol* 2008; 15(7): 1095-1105.
10. Bio-Rad. Platelia™ Aspergillus Ag test package insert. Reference 62794. Available at www.Bio-Rad.com.
11. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and update of the consensus definitions of invasive fungal disease from the European Organisation for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. *Clin Infect Dis* 2020; 71: 1367-1376.

12. Haydour Q, Hage CA, Carmona EM, et al. Diagnosis of fungal infections. A systematic review and meta-analysis supporting American Thoracic Society Practice Guideline. *Ann Am Thorac Soc* 2019; 16: 1179-1188.
13. Lass-Flörl C, Lo Cascio G, Nucci M, et al. Respiratory specimens and the diagnostic accuracy of aspergillus lateral flow assays (LFA-IMMY™): real-life data from a multicentre study. *Clin Microbiol Infect* 2019; 25(12): 1563.e1-1563.e3.
14. IMMY sōna Aspergillus Galactomannan LFA for the detection of Aspergillus Galactomannan. Ref AF2003. Package insert, revision date 24-07-2019, revision 5. Available at www.immy.com.
15. Jenks JD, Mehta SR, Taplitz R, et al. Bronchoalveolar lavage Aspergillus Galactomannan lateral flow assay versus Aspergillus-specific lateral flow device test for diagnosis of invasive pulmonary Aspergillosis in patients with haematological malignancies. *J Infect* 2019; 78: 257-259.
16. Jenks JD, Mehta SR, Taplitz R, et al. Point-of-care diagnosis of invasive aspergillosis in non-neutropenic patients: Aspergillus galactomannan lateral flow assay versus Aspergillus-specific lateral flow device test in bronchoalveolar lavage. *Mycoses* 2019; 62: 230-236.
17. Pan Z, Fu M, Zhang J, et al. Diagnostic accuracy of a novel lateral-flow device in aspergillosis: a meta-analysis. *J Med Microbiol* 2015; 64: 702-707.
18. Miceli MH, Goggins MI, Chander P, et al. Performance of lateral flow device and galactomannan for the detection of Aspergillus species in bronchoalveolar fluid of patients at risk for invasive pulmonary aspergillosis. *Mycoses* 2015; 58: 368-374.
19. Hage CA, Carmona EM, Epelbaum O, et al. Microbiological laboratory testing in the diagnosis of fungal infections in pulmonary and critical care practice. An official American Thoracic Society Clinical Practice Guideline. *Am J Respir Crit Care Med* 2019; 200(5): 535-550.
20. Garnham K, Halliday CL, Rai NJ, et al. Introducing 1,3-Beta-D-glucan for screening and diagnosis of invasive fungal diseases in Australian high risk haematology patients: is there a clinical benefit? *Int Med J* 2020; doi:10.111/imj.15046. On-line ahead of print.
21. Pasqualotto AC, Xavier MO, Sanchez LB, et al. Diagnosis of invasive aspergillosis in lung transplant recipients by detection of galactomannan in the bronchoalveolar lavage fluid. *Transplantation* 2010; 90: 306-311.
22. Johnson MD, Lewis RE, Dodds Ashley ES, et al. Core recommendations for antifungal stewardship: a statement of the Mycosis Study Group Education and Research Consortium. *J Infect Dis* 2020; 222: S175-S198.
23. Mercier T, Dunbar A, de Kort E, et al. Lateral flow assays for diagnosing invasive pulmonary aspergillosis in adult hematology patients: A comparative multicentre study. *Med Mycol* 2020; 58(4): 444-452.
24. Scharmann U, Verhasselt HL, Kirchoff L, et al. Evaluation of two lateral flow assays in BAL fluids for the detection of invasive pulmonary aspergillosis: a retrospective two-centre study. *Mycoses* 2020; 63(12): 1362-1367.
25. Wheat LJ, Nguyen MH, Alexander BD, et al. Long-term stability at -20°C of Aspergillus galactomannan in serum and bronchoalveolar lavage specimens. *J Clin Microbiol* 2014; 52(6): 2108-2111.