

PROPOSAL: INTRODUCTION OF A NEW TEST

SUMMARY

Test description: Detection of *Aspergillus* (galactomannan) antigen

Sample type(s): serum and broncho-alveolar lavage fluid

Procedure: one-stage immunoenzymatic sandwich microplate assay

Accreditation warranted: no

Can be ordered by: specialists, no specific medical specialty

Estimated number of tests/year: 5500 (see Excel sheet)

Estimated evolution over the next five years: further increase

Proposed B value: B700

Estimated total budget/year: 76257 euro (see Excel sheet)

Current reimbursement of the test: In Belgium most centers apply the RIZIV code 552016 - 552020 B 250 "Opzoeken van infectieuze agentia met een immunologische techniek".

Estimated cost savings/year: less use of antifungal agents (cost savings by preemptive therapy as opposed to prophylactic therapy or empiric therapy)

Tests that become obsolete and can be withdrawn from reimbursement: none

Expected impact of (new) concurrent test ordering: none

Access for other disciplines: no

Year report (with positive result percentage) warranted: no

Nederlandstalige omschrijving prestatie

1. Aaabbb Opzoeken van antigeen van *Aspergillus* in bloed

Diagnoseregul: enkel bij patiënten met een gastheer factor ('host factor') zoals gedefinieerd door de internationale consensuscriteria van de EORTC-IFICG/NIAID-MSG en maximaal drie maal per week

2. cccddd Opzoeken van antigeen van *Aspergillus* in broncho-alveolair lavage vocht

Diagnoseregul: enkel bij patiënten met een gastheer factor ('host factor') zoals gedefinieerd door de internationale consensuscriteria van de EORTC-IFICG/NIAID-MSG

Franstalige omschrijving prestatie

1. aaabbb Recherche d'antigène d'*Aspergillus* dans le sang

Règle diagnostique : seulement chez les patients avec un facteur hôte ('host factor') comme définie dans les critères consensus internationale du EORTC-IFICG/NIAID-MSG et maximum trois fois par semaine

2. cccddd Recherche d'antigène d'*Aspergillus* dans le liquide du lavage broncho-alvéolaire

Règle diagnostique : seulement chez les patients avec un facteur hôte ('host factor') comme définie dans les critères consensus internationale du EORTC-IFICG/NIAID-MSG

CLINICAL/DIAGNOSTIC SCENARIO

Short description: Why is this test proposed for reimbursement?

Aspergillosis continues to be a serious and common opportunistic infection in immunocompromised subjects. Invasive aspergillosis (IA) occurs in 5-20% of individuals who undergo allogeneic stem cell transplantation, while a lower proportion occurs in solid organ allograft recipients. Furthermore, mortality remains high, above 50% in most studies. While the effect of the serious underlying disease has a profound impact on outcomes, delayed diagnosis contributes to mortality. Diagnosis of IA may be difficult. Although air-crescent and halo signs seen on radiographs or computed tomography (CT) scans suggest IA, they are neither specific nor sensitive, and often are not correctly identified. Often, definitive diagnosis by biopsy is not feasible because of coagulation abnormalities. Bronchoscopy to obtain specimens for cytology or culture may be possible in such patients, but the sensitivity for diagnosis is only about 50%. Because of these diagnostic difficulties, most practitioners rely on universal prophylaxis and on the early empirical use of antifungals, especially in neutropenic cancer patients and stem cell transplant recipients. However, a strategy of preemptive or diagnostic-driven antifungal therapy is being explored based on advances in imaging techniques and in fungal antigen detection methods.

Galactomannan is an *Aspergillus*-specific antigen that is hematogenously released by fungal hyphae during invasive growth. Antigen detection for diagnosis of IA was first reported in the late 1970s, and was made a reality by the production of monoclonal antibodies and creation of a standardized and reproducible assay in the early 1990s. Available in Europe for over 10 years and CE marked, the Platelia® *Aspergillus* antigen immunoassay, produced by Bio-Rad Laboratories (Marnes-La-Coquette, France), was cleared by the Food and Drug Administration (FDA) for diagnostic use in the USA in May 2003.

Galactomannan detection is included in the mycological criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) standard definitions for invasive fungal infections. These standard definitions were first published in 2002 and updated in 2008. The definitions assigned 3 levels of probability to the diagnosis of invasive fungal infection that develops in immunocompromised patients with cancer and in hematopoietic stem cell transplant recipients—namely, “proven,” “probable,” and “possible” invasive fungal infection. The category of proven invasive fungal disease can apply to any patient, regardless of whether the patient is immunocompromised, whereas the probable and possible categories are proposed for immunocompromised patients only. Proven invasive fungal infection required only that a fungus be detected by histological analysis or culture of a specimen of tissue taken from a site of disease. By contrast, probable and possible invasive fungal infections hinged on 3 elements—namely, a host factor that identified the patients at risk, clinical signs and symptoms consistent with the disease entity, and mycological evidence that encompassed culture and microscopic analysis but also indirect tests, such as antigen detection.

The Belgian reimbursement criteria for the ‘newer’ antifungal agents, namely lipid formulations of amphotericin B, caspofungin, voriconazole and posaconazole restrict the use of these agents for the treatment of invasive aspergillosis to proven or probably infections according the EORTC/MSG definitions. Because of the low sensitivity of culture and the impossibility to take a biopsy, galactomannan detection is often the only mycological tool to provide a probable diagnosis of invasive aspergillosis and thus to initiate treatment of this life threatening disease.

APPRAISAL

1) Analytical performance characteristics (analytical validation report)

1.1 *Preanalytical considerations (patient variables, sample stability)*

- Biological variation (*sampling time (f.i. drug monitoring)*): N/A
- Interferences (*medication, diet, ...*)

Piperacillin-tazobactam may cause false-positive results^{1, 2, 3, 4, 5, 6, 7, 8, 9}. Piperacillin is produced by *Penicillium* spp., which contains galactomannan in its cell wall. Carryover of galactomannan through production was proposed as the cause for false positivity in patients receiving this antibiotic^{3, 9}. Viscoli et al.³ reported that the galactomannan test was positive in 74% of patients receiving piperacillin-tazobactam versus 11% of those treated with other antibiotics. Several studies have documented that the majority of lots of piperacillin-tazobactam contain galactomannan^{4, 6, 8}. False-positive results also were noted in patients receiving amoxicillin-clavulanic acid^{7, 9, 10}. Galactomannan has not been detected in other antibiotics, including cephalosporins, carbapenems, macrolides, aminoglycosides, and quinolones^{5, 6}, and negative results have been observed in patients receiving such antibiotics⁵. A few approaches were proposed to reduce the problem with piperacillin-tazobactam. Ideally, galactomannan should be excluded from medications used for the treatment of patients at risk of invasive aspergillosis. Restriction of piperacillin-tazobactam use to the preengraftment period reduced the positivity rate from 45 to 5% at one institution³. Although others have suggested that sampling at the trough time for piperacillin might reduce galactomannan levels below the test cutoff⁶, galactomannan accumulates with repeated administration⁵ and may remain positive for up to 6 days after discontinuation of piperacillin⁸. Impaired renal function may delay the clearance of galactomannan.

Surmont¹¹ described that Plasma-Lyte solution (a buffered intravenous solution containing sodium gluconate) can produce false positive galactomannan assay reactivity. Gluconate is produced by fermentation of glucose in mold cultures, including *Aspergillus* and *Penicillium* spp.

- Patient variables (*age, risk factors, gender, ...*)

False-positive results may occur more frequently in children. Herbrecht et al.¹² reported a specificity of 48% in children versus 97.5% in adults. Others noted false-positive results in 83% of premature infants¹³. Hayden, however, reported the specificity to be 98.4% in pediatric cases using a cut-off of 0.5¹⁴. Challier et al.¹⁵ reported 100% specificity in children and adults, but the pediatric controls consisted of only 12 subjects, of which only 4 had conditions predisposing of to IA. Furthermore, for 8 pediatric cases of possible IA, the test was positive in each, raising concern about false positivity. Gangneux et al.¹⁶ noted false-positive results in an infant fed exclusively with milk formula and noted high concentrations of galactomannan in the milk. Another potential cause of reduced specificity in children is gastrointestinal colonization with organisms producing cross-reactive antigens¹⁷. *Bifidobacterium* spp. are present in high concentration in the intestines of neonates and produce lipoteichoic acid that is positive in the galactomannan assay.

- Sample stability (*preservation, transportation, storage, ...*)

Care is required to avoid contamination of specimens with mould during collection, processing, shipment, and testing. Unopened specimens may be stored at 2 to 8°C for 5 days prior to testing, but for only 48 h after they are opened: longer storage requires freezing at -

20°C. Specimens should be packaged in cold packs to avoid warming during shipping. Pereira et al.¹⁸ observed that 20% of serum samples retested after 4 years of storage showed lower reactivity to the ELISA galactomannan test. The influence of storage on the performance of galactomannan testing in patients with IA was also suggested in a recent review¹⁹, although no detailed information was provided.

- Sample type (*serum, swab, urine, ...*)

The best performance characteristics of galactomannan detection in serum were shown in the neutropenic patient patients because of the angio-invasive character of the disease in this patient group. Several studies have now shown that use of the assay on BAL fluid provides very high sensitivity and specificity, with high predictive values, when performed in appropriate hosts (see attachment 1). Because galactomannan is released primarily by growing hyphae, and not by conidia that are colonizing the airways, it is thought that detection of GM may actually provide better evidence of actual *Aspergillus* invasion (notable by growth of hyphae) as opposed to either culture or polymerase chain reaction³¹. Despite the fact that the galactomannan enzyme immunoassay is not cleared for use in fluids other than serum, many large centers in the US and Europe now routinely use this test in the BAL panel. Because galactomannan is water soluble, it should also be detectable in other fluid samples, including CSF, and pleural fluid. Studies evaluating the role of galactomannan when testing body fluids other than serum have been reviewed by Klont et al.²⁰. The central nervous system (CNS) is often involved in patients with disseminated aspergillosis, and antigen may be detected in cerebral spinal fluid (CSF)^{21, 22, 23, 24}. However, antigen was also detected in CSF from antigenemic patients without CNS involvement, presumably due to entry of blood into the CSF caused by a traumatic lumbar puncture¹⁷. Detection of antigen in the CSF in the absence of antigenaemia would provide compelling evidence of CNS aspergillosis in the appropriate clinical setting. Antigen detection in other tissues and fluids has been tested, but the accuracy in those specimen types has not been determined^{17, 25}. Overall, the experience with galactomannan detection in other body fluids than serum and BAL remains limited.

- Sample volume

300 µl of test specimen is required.

- Prevalence (*estimation of amount of positive test percentage*)

The expected prevalence of IA varies with the patient population; rates up to 20% have been reported in the high-risk category (neutropenia, hematological malignancy and allogeneic HSCT)^{26, 27}. In allogeneic HSCT recipients, the incidence of IA has increased during the 1990's and although recent evidence suggests that the incidence has at least stabilized, infection is still common, estimated to be 6 to 10% per allogeneic HSCT annually. Of note, some centres report much higher incidences than others, suggesting geographical influence in either the exposure to *Aspergillus* species, differences in host risks, or significant diagnostic bias.

In a retrospective study performed at a medical intensive care unit (Meersseman et al.), 5.9% of hospitalized patients had proven or probable invasive aspergillosis. Excluding patients with hematologic malignancies or cancer, the rate of proven or probable IA was 3.7%.

- Target population

- High-risk category: patients with neutropenia, hematological malignancy or allogeneic HSCT
- Autologous HSCT, solid-organ cancer, HIV infection, lung transplantation, systemic diseases requiring immunosuppressive therapy

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- Patients admitted to the intensive care with prolonged treatment with corticosteroids, COPD, liver cirrhosis, severe burns, prolonged stay at ICU, malnutrition

1.2 Analytical considerations (reproducibility, accuracy, correlation, linearity, reference range)

- (Im)Precision: see below
- Accuracy (bias): N/A
- Correlation with current method/ standard: N/A
- Reproducibility (*within run, between run*):

Inter-assay and Intra-assay variability for the Platelia *Aspergillus* EIA were determined in a study by the FDA using a panel of 6 pooled patient serum samples (one negative, one low positive, two positive, and two high positive) obtained from actual clinical trial sites. Each of the 6 panel members were tested in triplicate (x3) on 3 different days, on 1 lot, at 2 sites (total number of replicates at each site = 9). Each of the 6 panel members was tested in duplicate (x2) on 3 different days, on 1 lot, at a third site (total number of replicates at the third site = 6). One (I) operator performed all precision testing at each site. The data was analyzed according to the Clinical and Laboratory Standards Institute (CLSI), formerly known as the National Committee for Clinical Laboratory Standards (NCCLS). The mean optical density (OD) and mean index value, standard deviation (SD), percent coefficient of variation (%CV), within lot precision (intra-assay) and within site (inter-assay) precision for each panel member at each site are illustrated below in the following tables.

Site 1

Panel Member	Neg		Low Pos		Pos #1		Pos #2		High Pos#1		High Pos #2		Neg Control		CO Control		Pos Control	
	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index
N	9	9	9	9	9	9	9	9	9	9	9	9	3	3	6	6	3	3
Mean	0.052	0.09	0.445	0.74	0.702	1.17	0.931	1.563	1.227	2.06	2.887	4.83	0.046	0.08	0.606	1.00	2.216	3.67
Within Run (intra-assay) ¹ SD	0.002	0.00	0.022	0.03	0.059	0.09	0.044	0.08	0.051	0.09	0.089	0.17	N/A	N/A	0.02	0.03	N/A	N/A
%CV	N/A	N/A	4.8%	4.4%	8.4%	7.6%	4.7%	5.1%	4.2%	4.4%	3.1%	3.6%	N/A	N/A	3.7%	3.4%	N/A	N/A
Total (inter-assay) ² SD	0.036	0.04	0.051	0.08	0.070	0.14	0.044	0.25	0.058	0.29	0.169	0.58	N/A	N/A	0.102	0.03	0.317	0.12
%CV	N/A	N/A	11.5%	10.4%	10.0%	11.6%	4.7%	15.7%	4.7%	14.3%	5.9%	11.9%	N/A	N/A	16.9%	2.8%	14.3%	3.3%

Site 2

Panel Member	Neg		Low Pos		Pos #1		Pos #2		High Pos#1		High Pos #2		Neg Control		CO Control		Pos Control	
	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index
N	9	9	9	9	9	9	9	9	9	9	9	9	3	3	6	6	3	3
Mean	0.040	0.10	0.280	0.70	0.364	0.89	0.602	1.49	0.801	2.01	1.361	3.43	0.074	0.18	0.415	1.00	1.197	2.97
Within Run (intra-assay) ¹ SD	0.006	0.01	0.041	0.09	0.023	0.07	0.045	0.11	0.046	0.10	0.047	0.11	N/A	N/A	0.00	0.01	N/A	N/A
%CV	N/A	N/A	14.5%	13.0%	6.4%	7.6%	7.5%	7.1%	5.7%	4.8%	3.5%	3.2%	N/A	N/A	1.1%	1.1%	N/A	N/A
Total (inter-assay) ² SD	0.006	0.03	0.058	0.19	0.083	0.18	0.057	0.28	0.042	0.53	0.079	1.00	N/A	N/A	0.094	0.01	0.068	0.54
%CV	N/A	N/A	20.8%	27.0%	22.7%	19.8%	9.5%	18.7%	5.3%	26.5%	5.8%	29.2%	N/A	N/A	22.7%	0.9%	5.7%	18.2%

Site 3

Panel Member	Neg		Low Pos		Pos #1		Pos #2		High Pos#1		High Pos #2		Neg Control		CO Control		Pos Control	
	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index	OD	Index
N	6	6	6	6	6	6	6	6	6	6	6	6	3	3	6	6	3	3
Mean	0.049	0.10	0.388	0.81	0.652	1.36	0.830	1.73	1.158	2.41	2.378	4.96	0.059	0.12	0.480	1.00	1.652	3.45
Within Run (intra-assay) ¹ SD	0.003	0.01	0.009	0.02	0.082	0.17	0.068	0.14	0.094	0.20	0.126	0.25	N/A	N/A	0.028	0.06	N/A	N/A
%CV	N/A	N/A	2.4%	2.4%	12.5%	12.2%	8.2%	8.2%	8.1%	8.2%	5.3%	5.1%	N/A	N/A	5.8%	5.8%	N/A	N/A
Total (inter-assay) ² SD	0.012	0.03	0.078	0.13	0.068	0.15	0.104	0.25	0.082	0.15	0.111	0.34	N/A	N/A	0.028	0.04	0.056	0.23
%CV	N/A	N/A	20.0%	15.8%	10.5%	11.1%	12.5%	14.3%	7.1%	6.2%	4.7%	6.8%	N/A	N/A	5.8%	4.1%	3.4%	6.6%

N/A = not applicable

¹NCCLS EPS-A, Vol. 19, No. 2, Page 24, Equation (C2)

²NCCLS EPS-A, Vol. 19, No. 2, Page 25, Equation (C3) and Equation (C4)

- Reference range

There have been considerable efforts in establishing the appropriate galactomannan ELISA cut-off to maximize clinical sensitivity and specificity. The ELISA endpoint is a continuous variable and the optimal cut-off should be determined after defining the receiver–operator curve relation (i.e., the relation between sensitivity and 1–specificity).²⁸ The cut-off optical density (OD) index value of 1.5 initially recommended by BioRad and used in many early studies has been progressively revised downwards; a cut-off OD index value of 0.5 is now currently accepted by the US Food and Drug Administration (FDA), and also used in Europe.^{29, 30}

- Analytical range/Linearity:

The limit of detection is using the sandwich ELISA is lower (0.5~1 ng galactomannan/L) than that achievable using the latex agglutination (15 ng/L).³¹

- Turnaround time (TAT) (*POCT feasible, necessary, ...*)

Technician time averages about 4 hours per assay.

1.3 Quality issues

- CTL (clinical tolerance limits): N/A
- Procedures available: see instruction leaflet
- Follow-up internal quality control:

Positive, negative and cut-off controls are included in the test kit

- Does external quality control exist? (*WIV, commercial, ...*): no

2) Diagnostic performance

2.1 Sensitivity, specificity

- *What was used as gold standard*: proven IA, requiring demonstration of fungal elements in diseased tissue
- *Why is expected the test under investigation performing better than the existing gold standard (if there is one)*: proof often not feasible (autopsy-biopsy)
- *Health impact of false-positives and false-negatives* false positives: initiation of unnecessary treatment with antifungal agents, false-negatives: treatment will not be initiated or will be initiated later
- *Economic impact of false-positives and false-negatives* very difficult to calculate
- *Proportion of tests than cannot be interpreted, (are inhibited) and their impact on health and cost*: none

Several factors must be considered in analysis of literature on sensitivity and specificity of the test. Although numerous studies have been performed to determine the sensitivity and specificity of the assay in various patient populations, the results are variable.

First, the criteria used for diagnosis must be considered. Most studies use the consensus definition recommended by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infection Cooperative Group (EORTC/IFICG) and the National Institutes of Allergy and Infectious Diseases Mycoses Study Group (NIAID MSG)³². Cases are classified as proven, probable, and possible, based upon the level of certainty of the clinical, radiographic and laboratory findings.

Second is the study design. Was the study prospective or retrospective? Were the tests performed real-time or in batch after completion of the study? And were the results used in

classification of the cases, or was classification based solely upon histopathology and culture results? Ideally, cases should be enrolled prospectively into a protocol designed to assess the accuracy of the test using accepted criteria for diagnosis other than the test under evaluation. Less rigorous design allows for bias that may affect the outcome of the analysis.

Third, the cut-off for positivity must be considered. The European kit recommended a cut-off index of 1.5 until 2006, whilst the U.S. kit recommended 0.5 since its approval by the FDA. Several reports have analyzed the data at cut-offs different from those recommended by the manufacturers.

Fourth, the type of population studied affects the performance of the test. The incidence of IA is highest in SCT patients, followed by neutropenic patients with underlying hematologic malignancy, and then solid-organ transplantation (SOT). The accuracy of the test is generally greatest in populations with the highest rate of IA, namely, allogeneic SCT recipients. The age of the patient also may impact the performance of the assay. Others reported reduced specificity during the first few weeks following allogeneic SCT and reduced sensitivity for the late cases occurring during immunosuppression for chronic graft versus host disease (CGVHD).

Fifth, the purpose of testing must be considered. Most studies report experience using the test to monitor patients with underlying hematologic malignancy or SCT for development of IA, while only a few report its use for evaluation of suspected cases.

Sixth, in studies using the assay to monitor patients for the development of IA, the frequency of screening affects its performance. Sensitivity is improved by more frequent screening, typically twice weekly during the period of greatest risk.

Seventh, several medications may affect the accuracy of the test. Mold active antifungal prophylaxis or empiric therapy may reduce its sensitivity. If the lower (0.5) cut-off is used, prior antifungal therapy appears to have a minor effect on the sensitivity of the test^{33, 34}. Conversely, antibiotics produced by *Penicillium* spp. may contain galactomannan and cause false-positive results.

The diagnostic potential of the galactomannan assay on serum has been assessed in at least 30 prospective studies. The *sensitivity* for proven and probable IA varies between 27.5% and 100%. However, a direct comparison of the results of these different studies is problematic, because of the factors mentioned above.

In 2006 a meta-analysis was published³⁵. Overall, the sensitivity of the test was 0.71 (95% CI, 0.68–0.74), and the specificity was 0.89 (95% CI, 0.88–0.90) for proven cases. For proven and probable cases, the sensitivity was 0.61 (95% CI, 0.59–0.63), and the specificity was 0.93 (95% CI, 0.92–0.94). The main finding was that the assay is moderately useful for surveillance of IA in patients with hematological malignancy or hematological transplant recipients. The performance of the test dropped sharply for solid-organ transplant recipients, for whom it had poor sensitivity and specificity. However, the problem of study heterogeneity was also a problem in this meta-analysis.

For the results of published studies that evaluated performance of the BioRad GM EIA applied to BAL fluids from patients we refer to attachment.

2.2 Likelihood ratio's (LR):

Likelihood ratios are shown below (Maertens et al ³⁶):

Author	Year	Likelihood ratio of proven/probable IA		Diagnostic
		Positive GM test result (LHR+)	Negative GM test result (LHR-)	odds ratio (LHR+/ LHR-)
Bretagne	1997	4.34	-	-
Machetti	1998	4.41	0.30	14.7
Maertens	1999	18.6	0.07	265.71
Sulahian	2001	11	0.24	45.83
Ulusakarya	2000	13.8	0.32	43.12
Herbrecht	2002	4.57	0.73	6.26
Maertens	2002	10.62	0.16	66.37
Becker	2003	2.87	0.64	4.48
Platelia package insert	2003	7.36	0.21	35
Jarque	2003	22.33	0.34	65.67
Moragues	2003	25	0.96	26.04
Pazos	2003	44	0.12	366.66
Pinel	2003	25	0.96	26.04
Buchheidt	2004	33	0.67	49.25
Maertens	2004	48.5	0.03	1616.66
Marr	2004	2.07	0.62	3.33
Rovira	2004	22.33	0.34	65.67
Yoo	2005	3.90	0.17	22.94

2.3 NND (number needed to diagnose): N/A

2.4 Other

- ROC-curves (other methods)
- Are other results needed to interpret this lab test?

In Europe, results were presented by two major centres ³⁰ that have used the PA-EIA routinely as a screening assay in high-risk neutropenic patients for many years. As evidenced by the AUC of the ROC curves, the diagnostic accuracy (or performance) of the assay was high and was identical in both centers. This analysis presents supportive evidence that an OD index cutoff of 0.5—identical to the approved cutoff in the United States—can be used reliably to define a case of probable IA in this particular patient population.

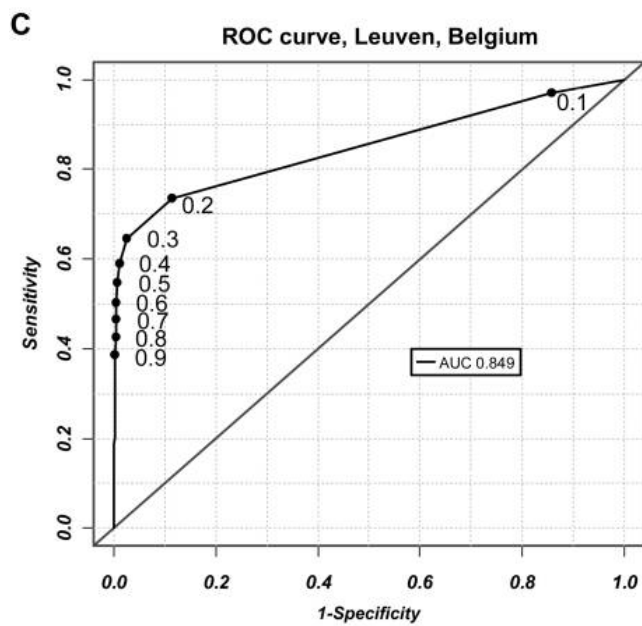
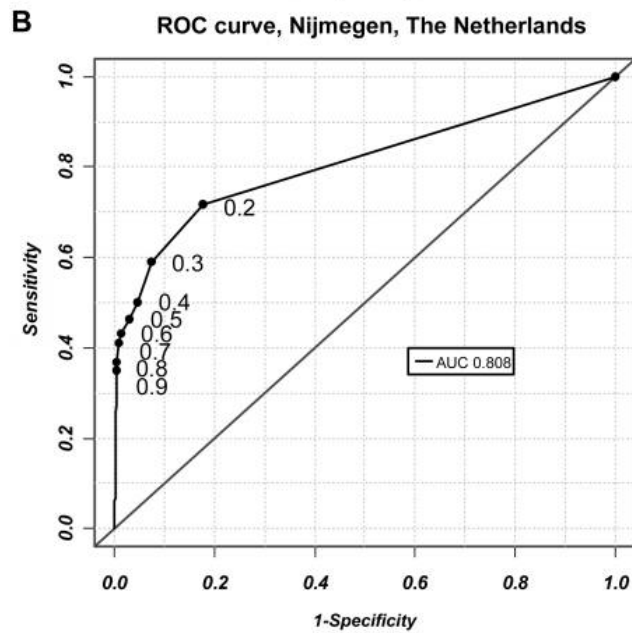
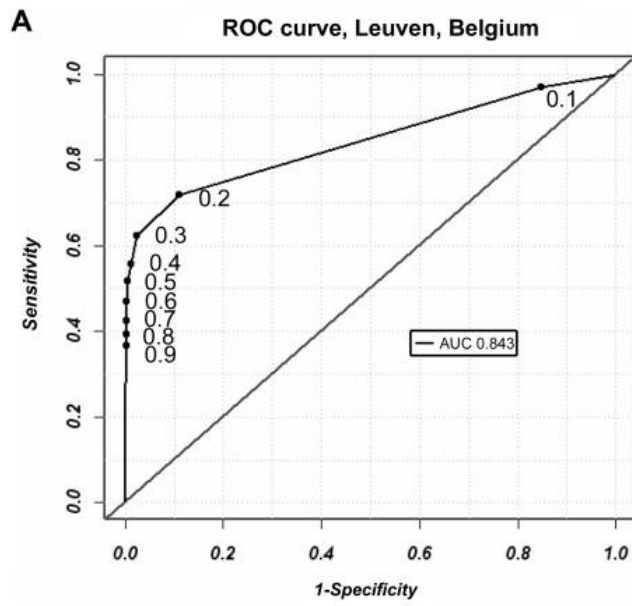
A high overall per-episode sensitivity of 97.4%, an acceptable specificity of 90.5%, and a high negative predictive value of 99.4% were achieved; the per-episode positive predictive value was only 66.1%, but it increased significantly (without affecting the other statistical parameters) to 87.5% when 2 consecutive samples with an OD index ≥ 0.5 were required to define positivity.

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Table 2. Sensitivity and specificity of the Platelia *Aspergillus* EIA (Bio-Rad Laboratories), according to selected cutoff optical density (OD) indices and European Organization for Research and Treatment of Cancer/Mycoses Study Group classification of episodes of invasive aspergillosis (IA).

OD index cutoff value, episode classification	No. of episodes with positive results/no. of episodes tested	Sensitivity, % (95% CI)	No. of episodes with negative results/no. of episodes tested	Specificity, % (95% CI)
OD index ≥ 1.5				
Proven IA	19/19	100 (85.4–100)
Probable IA	10/19	52.6 (28.9–75.5)
Overall	29/38	76.3 (59.8–88.6)
Control group	196/201	97.5 (94.3–99.2)
OD index ≥ 1.0				
Proven IA	19/19	100 (85.4–100)
Probable IA	12/19	63.2 (38.4–83.7)
Overall	31/38	81.6 (65.7–92.3)
Control group	194/201	96.5 (93.0–98.6)
OD index ≥ 0.5				
Proven IA	19/19	100 (85.4–100)
Probable IA	18/19	94.7 (74.0–99.9)
Overall	37/38	97.4 (86.2–99.9)
Control group	182/201	90.5 (85.6–94.2)
OD index $\geq 2 \times 0.5$				
Proven IA	19/19	100 (85.4–100)
Probable IA	16/19	84.2 (60.4–96.6)
Overall	35/38	92.1 (78.6–98.3)
Control group	196/201	97.5 (94.3–99.2)

The receiver operating characteristic (ROC) curves graphing sensitivity (true-positive results) versus 1-specificity (false-positive results) using multiple optical density index cut-off values to define positivity, are shown below. The optical density index cut-off value decreases from high to low values as the curves move from left to right. The performances of tests obtained in Leuven, Belgium, and in Nijmegen, The Netherlands, are shown in panels A and B. Panel C shows performance of the tests obtained in Leuven while mimicking a twice-weekly sampling frequency (identical to Nijmegen).



3) Clinical impact

3.1 *Diagnostic*

- *Can other (non)-laboratory examinations be avoided by this test?*
- *Does the test supply additional or more accurate information, not provided by other (non)-laboratory examinations?*

IA is a leading cause of death among immunocompromised patients, especially among those patients with hematological malignancy or those who undergo hematological or solid-organ transplantation. Clinical and radiologic diagnosis of IA has limited sensitivity and specificity³⁷. Diagnosis by histopathology or culture is limited by the need for invasive procedures and low sensitivity. For example, cytology was positive in only 23% and culture in 17% of patients with IA who underwent bronchoalveolar lavage³⁸. In patients with thrombocytopenia, a tissue diagnosis carries the risk of bleeding and is usually not advisable. The use of a biological marker as an adjunct for screening for IA in high-risk patients is attractive, because it is non-invasive and may detect evidence of IA prior to the appearance of clinical signs and symptoms.

3.2 *Treatment*

- *Does the test allow (faster) starting of adequate therapy (or can useless therapy be avoided)?*
- *Is there a better guidance of therapy by this test?*
- *Can toxicity be avoided?*
- *Does conditional reimbursement of medication exist, based on test results? (e.g. HSV and acyclovir)?*

Empirical antifungal therapy has been considered standard practice of care in neutropenic patients with fever that persists or recurs while they are receiving broad-spectrum antibiotics and has repeatedly been endorsed by consensus guidelines^{39, 40}. The aim is to ensure that patients with possible IA receive therapy early in the course of the disease, because early initiation of therapy seems to improve the survival rate. As a result, as many as 40%–50% of the high-risk neutropenic population may receive empirical antifungal therapy, whereas the true incidence of IA appears to be 10%–15%. Posaconazole, a triazole antifungal, has been recently shown to decrease IFI incidence and overall mortality in some high-risk patients compared with standard azoles (Cornely NEJM 2007, Ullman NEJM 2007)). Based on these data, European Conference on Infections in Leukemia (ECIL) guidelines currently recommend (AI recommendation) the use of posaconazole for prophylaxis of fungal infections in high risk patient groups. However, some critical issues have not yet been adequately addressed, including the generalizability of study results, impact of mucositis and gastrointestinal GVHD on drug bioavailability, need for therapeutic drug monitoring, impact of prophylaxis on the performance of diagnostic assays, and optimal treatment of breakthrough invasive fungal infections.

Overtreatment, as well as the negative effect of delaying therapy until disease is proven, could be overcome by a pre-emptive approach. Such a strategy targets the population in which there is sufficient evidence of pathogen invasion but no manifest symptomatic disease. Progress has been shown from the incorporation of non-culture-based microbiological techniques, including screening for circulating *Aspergillus* galactomannan with an EIA and the early use of high-resolution thoracic CT scanning (HRCT). Maertens

et al⁴¹ showed that antigenemia preceded diagnosis on the basis of radiologic examination or *Aspergillus* isolation by 8 and 9 days in 80% and 88.8% of patients, respectively. Antigenemia preceded therapy in 83.3% of patients. In a prospective feasibility study it was shown that a preemptive approach (based on galactomannan detection and HRCT scanning) led to a reduction of the rate of antifungal use by 78% and to the early initiation of antifungal therapy in 7.3% that were clinically not suspected of being an invasive fungal infection⁴².

Monitoring for antigen clearance or rebound may provide useful information for assessing the effectiveness of therapy. Bretagne et al.⁴³ and Maertens et al.⁴⁴ described declining levels in patients who responded to therapy and rising concentrations in those with fatal outcomes. Rohrllich et al.⁴⁵ reported clearance of antigenemia in patients who responded to therapy and reappearance in those who relapsed. Boutboul et al.⁴⁶ noted stable levels in responding patients and rising levels in failing patients. Becker et al.⁴⁷ observed that antigen was no longer detectable in BAL after three days of therapy and others have reported a decline in CSF antigen concentration with therapy^{21,22}.

Monitoring antigenemia may assist in patient management. Failure of antigenemia to decline would suggest treatment failure, and support consideration of modifying therapy, including combination therapy⁴⁸. Conversely, clinical deterioration in the presence of falling antigen levels would support investigation of other causes for the clinical deterioration. Rebound antigenemia after treatment was stopped suggests relapsing infection and the need for resumption of therapy.

The reimbursement of several antifungal agents (lipid formulations of amphotericin B, voriconazole, posaconazole and caspofungin) is based on the EORTC criteria in which galactomannan detection is incorporated as a mycological criterion.

3.3 Health outcome

- *Can illness, complications, morbidity, mortality be prevented?*

Von Eiff showed in his study that early diagnosis of pulmonary aspergillosis improves survival⁴⁹. Initiation of antifungal treatment later than 10 days after the onset of pneumonia resulted in a mortality of 90%, as opposed to 41% with an earlier start of antimycotics ($p < 0.01$).

Abandoning empirical antifungal therapy for a pre-emptive approach spares patients from exposure to expensive and potentially toxic drugs. Alternatively, the pre-emptive approach offered effective antifungal control for patients with invasive aspergillosis, even for those not qualifying for empirical antifungal therapy. A randomized trial comparing outcome and cost effectiveness of fever-driven empirical therapy versus an EIA or CT-driven pre-emptive approach may be in order.

3.4 Other:

- *Are there epidemiological interests to perform this test? Outbreak monitoring?*
no
- *Is the test still in research faze? No, more than 10 years experience*

4) Organizational impact (no data available)

4.1 Impact in the hospital

- *Length of stay, ...*

4.2 Impact outside the hospital

- *Patient transportation (POCT, ...)*

5) Cost impact: in and outside the laboratory

5.1 (Activity-Based) Cost/test (reagents, personnel, overhead (housing, QC, ...))

- R&D cost if applicable (in-house testing)
- How many reagent kits have been sold?
- See also Excel template

The total cost per galactomannan test including both direct and indirect costs (lab and hospital overhead) is 16 euro/test.

5.2 Reimbursement

Can other tests be withdrawn? No. Most centers currently apply the RIZIV code 552016 - 552020 B 250 “Opzoeken van infectieuze agentia met een immunologische techniek”. This will not be the case if reimbursement for galactomannan is available.

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5.3 Profit elsewhere in the hospital

To date, no cost studies have been conducted, but a hypothesis can be supported that by permitting early diagnosis, these tests may be cost-effective. Wilson et al.⁵⁰, for example, noted an attributable cost of \$87,000 per case of IA in transplant patients. Musher et al.³³ noted that positive antigen results in BAL specimens may have averted the need for additional bronchoscopy or lung biopsy in up to 90% of cases with negative cultures and histopathology following an initial bronchoscopy, possibly reducing cost and morbidity. Severens et al.⁵¹ explored the cost-effectiveness of a strategy using antigenemia testing to monitor neutropenic patients, whereby testing could reduce the use of empiric antifungal therapy. Although only 10% of neutropenic patients with fever, despite receiving broad-spectrum antibiotics, have an invasive fungal infection, over half receive antifungal therapy. The cost of empirical treatment with caspofungin comes to ~ 483 euro per day (or 3381 euro/week) whereas the cost of a screening with galactomannan during one week (thrice weekly testing) is about 40 euro. Using a prophylactic treatment strategy with posaconazole in patients with neutropenia, all patients are treated during the period of neutropenia at a cost of ~ 2800 euro's (4 flacons) per episode. Of course many factors need to be included in cost-benefit analysis and a thorough evaluation of cost-benefit is certainly warranted.

6. Decision making

6.1 *Impact on the clinical decision making process and patient management*

See 3.2

6.2 *Overexploitation/underutilization*

6.3 *Incorporated in Clinical Practice Recommendations/Guidelines?*

Galactomannan detection is included in the mycological criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (MSG) standard definitions for invasive fungal infections.

COMMENTS

TO DO/ACTIONS

ATTACHMENTS

Results of published studies that evaluated performance of the BioRad GM EIA applied to BAL fluids from patients are summarized below

Author, yr	Study design	Population, number cases / controls	Reference standard used	ELISA test index cutoff	Sensitivity Specificity PPV, NPV ROC	General conclusions	Limitations
Meersseman, 2007 ⁵²	Prospective cohort	110 total (33% heme malignancy, 67% other) Cases: 26 proven Controls: 43	Modified EORTC / MSG criteria ¹	0.5	Sensitivity 88% ¹ Specificity 87% ROC AUC 0.898	High SN, SP No impact of pip-tazo on specificity Culture sensitivity 58% Higher GM EIA values in patients with proven IA vs. less diagnostic certainty Higher sensitivity compared to serum assay	No data on influence of antifungal therapy Population in ICU (severely ill)
Nguyen, 2007 ⁵³	Retrospective cohort	73 patients (non-immunosuppressed) Cases: 6 proven Controls: 67	Modified EORTC / MSG criteria ²	1.0	Sensitivity 100% Specificity 88.1% PPV 42.9% NPV 100%	Relatively low PPV due to low prevalence in non-immunosuppressed population High NPV	Low prevalence population tested Small number of cases
Husain, 2007 ⁵⁴	Retrospective cohort	116 patients (lung transplant recipients) Cases: 6 (2 proven, 4 probable) Controls: 100	Modified EORTC / MSG criteria ²	1.0	Sensitivity 60% Specificity 95% ROC AUC 0.938	Useful test in lung transplant recipients when applied with higher (1.0) index cut-off	Small number of cases
Clancy, 2007 ⁵⁵	Retrospective cohort	81 patients (heart, kidney, liver, lung transplant recipients) Cases: 5 (2 proven, 3 probable)	Modified EORTC / MSG criteria ²	1.0	Sensitivity 100% Specificity 90.8% PPV 41.7% NPV 100%	Sensitivity better than with use of conventional tests (culture, histopathology, serum GM EIA) Use leads to more rapid diagnosis False positive results predominantly in lung transplant recipients	Small number of cases Population heterogeneity with low prevalence of infection

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Musher, 2004 ⁵⁶	Case / Control	All hematological malignancies Cases: 50 (22 proven, 28 probable) Controls: 50	EORTC / MSG criteria	0.5	Sensitivity 76% Specificity 94% PPV 84% ³ NPV 96% ³	Good performance in population with hematological malignancies Highest sensitivity in culture-positive BAL samples (89%); relatively high sensitivity in culture-negative BAL samples (59%)	Retrospective study that utilized previously frozen samples
Sanguinetti, 2003 ⁵⁷	Retrospective cohort	All hematological malignancies Cases: 20 (5 proven, 15 probable)	EORTC / MSG criteria	1.5	Sensitivity 100%	High sensitivity	Did not report specificity
Becker, 2003 ⁵⁸	Retrospective cohort 2 nd validation study	All hematological malignancies Cases: 17 Controls: 143 Validation study: Cases: 22 Controls 176	EORTC / MSG criteria	1.0	Sensitivity 100% Specificity 100% PPV 100% NPV 100% Validation study: Sensitivity 85% Specificity 100% PPV 100%, NPV 88%	Better performance with BAL analysis compared to serum	Retrospective study that utilized previously frozen samples Only neutropenic patients
Verweij, 1995 ⁵⁹	Retrospective cohort	All hematological malignancies Cases: 7 (probable) ⁴ Controls: 10 Non-neutropenic controls= 35	Probable: radiograph with "classic" abnormality (cavity)	OD at 1.0 ng GM (index not utilized)	Sensitivity 71% Specificity 100%	Can detect GM in BAL of patients considered to have IA	Small sample Did not vary index values for GM EIA cut-off Did not utilize standard definitions of IA

OD, Optical Density; AF, Antifungal Therapy; EORTC/MSG, European Organization for Research and Treatment of Cancer / Mycosis Study Group; SN, Sensitivity; SP Specificity; PPV, Positive Predictive Value; NPV Negative Predictive Value; ROC, Receiver Operator Curve; AUC, Area Under Curve; GM, Galactomannan; EIA, Enzyme Immunoassay; IA, Invasive Aspergillosis; ICU, Intensive Care Unit

¹ Modified to include COPD, cirrhosis and receipt of corticosteroids in host criteria. 27 patients with "possible" IA and 8 patients with "probable" IA eliminated from analysis due to diagnostic uncertainty.

² Modified to exclude host criteria (in populations that do not include cancer patients)

³ Case – control study. PPV, NPV estimates assume 20% prevalence of proven / probable IA in population that undergoes BAL

⁴ 2 patients with "possible" IA, defined as having radiographic abnormalities that are not considered 'classic' for IA were eliminated from analysis due to diagnostic uncertainty

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