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ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: diagnostic procedures

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Abstract

As the mortality associated with invasive *Candida* infections remains high, it is important to make optimal use of available diagnostic tools to initiate antifungal therapy as early as possible and to select the most appropriate antifungal drug. A panel of experts of the European Fungal Infection Study Group (EFISG) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) undertook a data review and compiled guidelines for the clinical utility and accuracy of different diagnostic tests and procedures for detection of *Candida* infections. Recommendations about the microbiological investigation and detection of candidaemia, invasive candidiasis, chronic disseminated candidiasis, and oropharyngeal, oesophageal, and vaginal candidiasis were included. In addition, remarks about antifungal susceptibility testing and therapeutic drug monitoring were made.

Keywords: Biomarkers, *Candida*, diagnosis, guideline, noncultural

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Introduction

One of the main novelties of the ESCMID *Candida* Guidelines is the inclusion of recommendations about diagnostic procedures. The aim of these guidelines is to appraise the different techniques and procedures for detection and investigation of *Candida* infections. Timing of antifungal therapy has been shown to have major impact on hospital mortality. As the mortality associated with invasive *Candida* infections remains high, it is important to make optimal use of diagnostic tools to initiate antifungal therapy as early as possible with the best antifungal drug. In addition to diagnostic tools understanding of the local epidemiology, patient risk factors and resistance profiles of *Candida* species are essential. In some geographical areas, the number of patients with candidiasis is rising associated with an increase in the number of patients with immunosuppression and the expanding utilization of intensive care units. New diagnostic utilities are being implemented. Most of the new detection methods have been designed to diagnose invasive candidiasis and have been shown to be valuable techniques, which could detect infection early.

This article includes recommendations about conventional methods of microbiological diagnosis of deep-seated, oropharyngeal, oesophageal and vaginal candidiasis, antifungal susceptibility testing (AST) and alternative diagnostic procedures also known as nonculture, biomarker detection procedures. Some issues about therapeutic drug monitoring (TDM) of antifungal agents are also commented upon.

Clinicians often use diagnostic tests as a package or strategy based on evidence regarding the accuracy of procedures. Several proposals have been published for grading quality of evidence and strength of recommendations for diagnostic tests and strategies [1]. Although recommendations on diagnosis share the fundamental logic of recommendations for other interventions, they present unique aspects. Conventional diagnostic procedures such as microscopical examination, culture and identification of microorganisms are essential investigations, and their performance depends on the possibility of obtaining samples of deep tissues. Consequently, grading the quality of evidence and strength of recommendation for conventional methods of diagnosing candidiasis has not been included in this guideline.

However, strengths of recommendations about new nonculture-based techniques for biomarker detection can be assigned because many techniques are available showing different levels of accuracy. The use of tests to establish the presence or absence of the disease and their utility as early diagnostic methods can be also evaluated. Table 1 shows the system used in these guidelines for grading quality of evidence about the accuracy of biomarker detection procedures in the diagnosis of candidiasis.

TABLE 1. System used in these guidelines for grading quality of evidence about the accuracy of biomarker detection procedures in the diagnosis of candidiasis (based on reference 1)

Accuracy[a]	
Highly recommended	Technique is accurate in >70% of cases (most)
Recommended	Technique is accurate in 50–70% of cases (reasonable number)
Not Recommended	Technique is accurate in <50% of cases (small number)
No recommendation	No data
Quality of evidence accepted	
Level I	Evidence from at least one properly designed prospective multicentre cross-sectional or cohort study
Level II	Evidence from (i) at least one well-designed prospective single-centre cross-sectional or cohort study or (ii) a properly designed retrospective multicentre cross-sectional or cohort study or (iii) from case-control studies
Level III	Opinions of respected authorities, clinical experience, descriptive case studies or reports of expert committees

[a]Accuracy was defined as: (Numbers of true positives + true negatives) divided by (Numbers of true positives + false positives + false negatives + true negatives).

This document was written by a panel of experts of the European Fungal Infection Study Group (EFISG) of the ESCMID. The text is divided into seven sections, and the object of the experts was to draw up a series of practical recommendations, with the aim of answering all the questions faced by health professionals when designing diagnostic strategies for detecting *Candida* infections.

1. What are the best tests for diagnosing candidaemia?

Candidaemia can be defined as the presence of any species of the genus *Candida* in the blood. Subsequently, blood cultures (BC) are essential for diagnosing candidaemia [2]. There are a number of international guidelines including general recommendations for taking and processing of blood samples to ensure the optimal isolation of microorganisms [3–6].

The number of BC recommended in a single session is 3 (2–4), with a total volume varying according to the age of the patient, 40–60 mL for adults, 2–4 mL for children under 2 kg, 6 mL between 2 and 12 kg, and 20 mL between 12 and 36 kg. The timing for obtaining the BC is one right after the other from different sites, and venipuncture remains the technique of choice. A BC set comprises of 60 mL blood for adults obtained in a single session within a 30-min period and divided in 10-mL aliquots among three aerobic and three

anaerobic bottles. The frequency recommended is daily when candidaemia is suspected, and the incubation period must be at least 5 days.

When these recommendations have been followed the sensitivity of BC to detect *Candida* is 50–75% although lower sensitivity rates in neutropenic patients and those undergoing antifungal treatment have been reported [7,8]. Some other remarks should be noted. Sensitivity varies depending on the species and system used. For instance, *C. glabrata* grows less optimally in the BACTEC™ medium (Becton Dickinson Diagnostic Systems) unless a mycosis bottle is included [7,8]. Identification to species level is mandatory because antifungal therapy can vary according to *Candida* species. In addition, yeasts in BC are not always *Candida* as other emerging and rare yeast pathogens have been involved in up to 5% of patients with fungemia. Lysis-centrifugation procedures showed higher efficacy when older BC systems were used as comparators. The recommendation of the panel was to use an automated validated BC system.

The performance of BC is not very high, and they cannot be considered as early diagnostic techniques. Alternative procedures based on the detection and quantification of fungal biomarkers and metabolites have been developed to improve and anticipate the detection of candidaemia. Table 2 includes the recommendations of the panel about the clinical use of these techniques.

The combined detection of mannan and anti-mannan antibodies is considered to be a method for specific detection of *Candida* spp. in serum samples [9]. There is a combination of tests available [Platelia Candida Antigen Plus (Ag Plus™) and Antibody Plus (Ab Plus™; Bio-Rad Laboratories)]. A number of studies, based on previous generations of these tests, reporting evidences from properly designed retrospective multicentre cross-sectional or cohort study and from case–control studies have proven their efficacy in the diagnosis of candidemia, with sensitivity and specificity rates around 80% and 85%, respectively, which translates into an accuracy of 50–70%. Serial determinations may be necessary. These assays can help to detect the infection early because they can be positive 6 days on average prior blood cultures. It shows also very high negative predictive value (>85%) and can be used to rule out infection. The panel considered the method as *recommended* for the diagnosis of candidaemia. It could be used as part of a diagnostic strategy to establish

TABLE 2. Summary of recommendations by *Candida* disease, specimen and test evaluated

Disease	Specimen	Test	Recommendation	Level of evidence
Candidaemia	Blood	Blood culture	Essential investigation[a]	NA
	Serum	Mannan/anti-mannan	Recommended	II
		B-D-glucan	Recommended	II
		Other antibodies	No recommendation	No data
		Septifast PCR kit	No recommendation	No data
		In-house PCR	No recommendation	No data
Invasive candidiasis	Blood	Blood culture	Essential investigation	NA
	Serum	Mannan/anti-mannan	No recommendation	No data
		B-D-glucan	Recommended	II
		Septifast PCR kit	No recommendation	No data
		In-house PCR	No recommendation	No data
	Tissue and sterile body fluids	Direct microscopy and histopathology	Essential investigation	NA
		Culture	Essential investigation	NA
		Immuno-histochemistry	No recommendation	No data
		Tissue PCR	No recommendation	No data
		In situ hybridization	No recommendation	No data
Chronic disseminated candidiasis	Blood	Blood culture	Essential investigation	NA
	Serum	Mannan/anti-mannan	Recommended	II
		B-D-glucan	Recommended	II
		Septifast PCR kit	No recommendation	No data
		In-house PCR	No recommendation	No data
	Tissue and sterile body fluids	Direct microscopy and histopathology	Essential investigation	NA
		Culture	Essential investigation	NA
		Immuno-histochemistry	No recommendation	No data
		Tissue PCR	No recommendation	No data
		In situ hybridization	No recommendation	No data
Oropharyngeal and oesophagic candidiasis	Swab	Culture	Essential investigation	NA
		In-house PCR	No recommendation	No data
	Biopsy[b]	Direct microscopy and histopathology	Essential investigation	NA
		Culture	Essential investigation	NA
		In-house PCR	No recommendation	No data
Vaginal candidiasis	Swab/vaginal secretions	Direct microscopy	Essential investigation	NA
		Culture	Essential investigation	NA
		Commercial tests	Use validated test only	NA
		In-house PCR	No recommendation	No data

NA, not applicable.
[a]Essential investigation means it must be done if possible.
[b]Oropharyngeal biopsy is not mandatory.

the absence of the disease to reduce the unwarranted use of antifungal agents in prophylactic and empirical regimens in critical care settings (ICU).

The β-1,3-D-glucan detection (BDG) is also a technique useful for *Candida* detection. It is not specific for *Candida* because it is present in many fungal species. The BDG test is considered to be a panfungal diagnostic method and was included in the EORTC/MSG (European Organization for Research and Treatment of Cancer/Mycosis Study Group) diagnostic criteria for invasive fungal infections in 2008, for all types of patients. There are several techniques on the market for the detection of glucan in serum. In Europe and America, the most used is Fungitell® (Associated of Cape Cod, Inc.). A number of meta-analyses have been undertaken using data from cross-sectional, cohort and case–control studies on the diagnosis of candidaemia. The sensitivity of glucan detection was >65% in most studies with a cut-off value of 80 pg/mL, with specificity rates >80%, positive likelihood ratios approximately of 4, negative likelihood ratios of 0.50 and negative predictive values >85%. The use of albumin, gauzes, immunoglobulins or haemodialysis was associated with false positives, and the test seemed of greater utility in patients who did not have haematological diseases such as surgical or medical ICU patients suffering from *Candida* infections [10]. The panel considered the BDG test (Fungitell™ only so far) as *recommended* for candidemia detection in adults being also very useful for ruling out infection. Serial determinations (twice a week) are recommended. The test has not been validated in children.

Regarding other alternative methods, the panel did not make any recommendations because no data are available to evaluate their utility for the clinical diagnosis of candidaemia. Antibody detection kits such as Serion Elisa Classic® and *Candida* germ tube antibodies are under evaluation, and there are limited data about their clinical accuracy. Molecular detection techniques largely PCR-based have also been designed, and several studies about their reliability are in progress. The Light Cycler SeptiFast® system (Roche) is a PCR-based commercial kit to detect bacteria and fungi in blood samples. Studies have reported some cases of candidaemia being detected by this kit, but the number of cases is rather limited and no recommendation can be made [11–13]. Regarding in-house PCR techniques, many reports have been published including more than 1000 patients [14–17]. Their pooled sensitivity and specificity was calculated over 85% in a meta-analysis published recently [18]. None of the PCR techniques included external validation and different material and methods were used. Third-party appraisal of results and harmonization of PCR-based techniques should be made before recommendations can be made regarding clinical utility.

2. What are the best tests for diagnosing invasive candidiasis?

Invasive candidiasis (IC) can be defined as a deep-seated disease, frequently a multiorgan infection including candidaemia although BCs are negative in as many as one-third of the cases at least in the ICU population [19]. Remarks about BC were made in the previous section. This section relates the recommendation by the panel about IC diagnosis using other specimens and procedures.

Classical diagnostic methods, such as direct microscopy, histopathology and culture, exhibit a limited sensitivity to detect IC, and their usefulness depends on the possibility of obtaining samples of deep tissues which, in many cases, cannot be taken due to the patient's condition. Therefore, these approaches must be considered as essential investigations to be performed if possible [3,5,6,20].

A number of considerations and recommendations were highlighted by the panel about the classical methods. Regarding tissue samples and body fluids from normally sterile sites, they must be obtained and collected aseptically and transported to the laboratory promptly. Small samples are prone to sampling error. Tissue for histopathology should be placed in fixative as rapidly as possible, and microscopy should include special stains such as silver stains and PAS. The use of optical brighteners is recommended for microscopical examination of un-fixed specimens. Microscopic examination requires expertise for interpretation, and morphology cannot be used for definitive identification [21–23].

Samples for culture should not be placed in histopathology fixatives and must be kept moist. They have to be processed promptly to avoid multiplication of organisms. If not possible, storage at 4–5°C is recommended. Fungal selective media must be included, and it should be observed that some species take several days (5–14 days) to grow in culture. Yeast isolation from normally sterile tissues or fluids is usually indicative of deep-seated infection. Negative culture results do not exclude *Candida* infection. Identification of the isolate to species level is mandatory [24,25].

Samples from tissues and body fluids can be also investigated using alternative procedures. Among these, immunohistochemistry [21–23], *in situ* hybridization [26] and analysis of samples by PCR-based procedures [15,27] have been positively evaluated in some studies, but they are not generally available and third-party evaluation of their accuracy has not been carried out so far. However, some general comments can be made. PCR-based procedures must use free DNA materials, and their performance may improve if they are

carried out following laser microdissection [28]. Immunohistochemistry has shown clinical utility to confirm infection when yeasts have been seen in tissue and BCs were negative. The panel recommended genus-specific antibody commercially available only (e.g. Rabbit anti *C. albicans*, type A:Biotin®, Serotec, No. 1750-5557). It should be noted that only positive results are reliable and negative results do not exclude the disease. Regarding *in situ* hybridization and tissue and body fluid PCR, there are no clinically validated commercially available kits to detect fungal infections.

Detection of IC by quantification of fungal components in body fluids other than serum has not been evaluated. However, there are some reports including cases of IC and quantification of serum biomarkers, but significant findings were reported for the BDG test only [10]. According to these results, the BDG test can be *recommended* for IC detection similar to that recommendation made for candidaemia detection (Table 2).

3. What are the best tests for diagnosing chronic disseminated candidiasis?

The same recommendations made for BC, tissue and body fluid samples for the detection of IC (Table 2) can be considered for diagnosing chronic disseminated candidiasis (CDC). The panel remarked, however, that a tissue biopsy is highly advisable because CDC is rarely detected by BC. In addition, the detection of biomarkers can be useful. As for IC, the BDG test has shown to be strongly associated with clinical findings and the panel considered the test as *recommended* for CDC detection [10]. Chronic disseminated candidiasis can be diagnosed by mannan and anti-mannan quantification. A meta-analysis mentioned previously suggests that the technique is very useful in CDC cases [9]. The report included 21 cases of CDC and mannan and anti-mannan quantification test exhibited 86% of sensitivity rate. Positive results were seen 16 days in average prior to cultures.

4. What are the best tests for oropharyngeal candidiasis and oesophagitis?

The essential specimen for the detection of those diseases is a swab taken from the lesion. A biopsy is not mandatory (Table 2), but it might discriminate between infection and colonization. Swabs must be inoculated on selective media to avoid overgrowth by colonizing bacteria. Species identification and susceptibility testing are recommended in recurrent/complicated cases and in patients who have been exposed to azoles previously. When a biopsy is obtained, it must be processed according to recommendations stated in the IC diagnostic procedures section. PCR-based methods have been evaluated, but no recommendation can be made as results have not been validated in a clinical setting [5,29,30].

5. What are the best tests for *Candida* vaginitis?

Examination of swabs and vaginal secretions is very valuable in detecting this infection (Table 2). A swab is less useful for microscopy than secretions. Vaginal secretions spread directly onto a microscopy slide, and left to dry is recommended. The observation of pseudohyphae can help to detect the infection, but filaments can be observed in patient without infection. In addition, not all *Candida* spp. form filaments during infection (e.g. *C. glabrata*), and microscopy in such cases will show only yeast cells [31].

Culture of swabs and vaginal secretions are also essential investigations. Semi-quantitative techniques using fungal selective agar are recommended. Species identification and susceptibility testing are indicated in recurrent/complicated cases and in patients with prior azole exposure.

Commercial tests designed to detect vaginal candidiasis can be also used, but the panel recommended the use of validated tests only [32,33]. PCR-based procedures have not been validated, and no recommendations can be made [34].

6. When are AST recommended for patient management and when for epidemiological reasons?

Recommendations for AST were also made by the panel. The panel considered that AST must be recommended for patient management for all *Candida* strains isolated from blood and other deep sites. Experts advised that reference procedures [35–39] or validated commercial techniques should be used [40–43]. However, it should be noted that discrepant results may be obtained with commercial techniques (such as Etest™ and Sensititre YeastOne™) as compared to the reference methods particularly for isolates with borderline MIC values. Importantly, interpretation of AST results requires expertise and cautious evaluation. It is essential to ensure the endpoints generated for each species mirrors those of reference methods before reference breakpoints are adopted for interpretation of results by commercial techniques. Antifungal susceptibility testing can be useful particularly in some cases such as strains from patients exposed to antifungal agents, isolates from patients

with clinical failure, strains belonging to rare and emerging species and species that are known to be resistant or less susceptible to antifungal drugs [44,45].

Regarding superficial isolates, AST can be recommended for patient management in cases who failed to respond to antifungal agents or relapsing infection. Surveillance cultures from patients exposed to antifungal agents could be also useful.

For epidemiological reasons, the panel recommended that all isolates from blood and deep sites should be tested using a reference method. Periodical epidemiological studies should be carried out including strains isolated from superficial sites to determine the susceptibility profiles and resistance rates for each individual centre [44,45].

Table 3 shows breakpoints to interpret AST results approved by both the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical Laboratory Standards Institute (CLSI) [46–53].

7. Is therapeutic drug monitoring indicated for patient management?

The panel indicated that TDM must be used for patients treated with 5-fluorocytosine. In addition, TDM is not normally required for drugs used (fluconazole, echinocandins and amphotericin B formulations) in the treatment for *Candida* infections except for patients with extra-corporeal membrane oxygenation (ECMO) treated with echinocandins as it can reduce the level of the antifungal being used [54–57].

Therapeutic drug monitoring is recommended if voriconazole or posaconazole is prescribed, and monitoring is highly recommended in unsatisfactory response to therapy, suspicion of toxicity or drug interaction(s), impaired liver or renal function and also in patients on ECMO [58–60].

TABLE 3. Interpretative breakpoints of antifungal agents approved by EUCAST and CLSI for susceptibility testing of *Candida*

		EUCAST			CLSI			
Antifungal	**Species**	**Susceptible**	**Intermediate**	**Resistant**	**Susceptible**	**S-DD**	**Intermediate**	**Resistant**
Amphotericin B	*C. albicans*	≤1	–	>1	NEY	NEY	NEY	NEY
	C. glabrata	≤1	–	>1	NEY	NEY	NEY	NEY
	C. krusei	≤1	–	>1	NEY	NEY	NEY	NEY
	C. parapsilosis	≤1	–	>1	NEY	NEY	NEY	NEY
	C. tropicalis	≤1	–	>1	NEY	NEY	NEY	NEY
Itraconazole	*C. albicans*	NEY	NEY	NEY	≤0.12	0.25–0.50	–	≥1
	C. glabrata	NEY	NEY	NEY	≤0.12	0.25–0.50	–	≥1
	C. krusei	NEY	NEY	NEY	≤0.12	0.25–0.50	–	≥1
	C. parapsilosis	NEY	NEY	NEY	≤0.12	0.25–0.50	–	≥1
	C. tropicalis	NEY	NEY	NEY	≤0.12	0.25–0.50	–	≥1
Fluconazole	*C. albicans*	≤2	4	>4	≤2	4	–	≥8
	C. glabrata	IE	IE	IE	–	≤32	–	≥64
	C. krusei	PT	PT	PT	PT	PT	PT	PT
	C. parapsilosis	≤2	4	>4	≤2	4	–	≥8
	C. tropicalis	≤2	4	>4	≤2	4	–	≥8
Voriconazole	*C. albicans*	≤0.125	–	>0.125	≤0.12	–	0.25–0.50	≥1
	C. glabrata	IE	IE	IE	IE	IE	IE	IE
	C. krusei	IE	IE	IE	≤0.50	IE	1	≥2
	C. parapsilosis	≤0.125	–	>0.125	≤0.12	–	0.25–0.50	≥1
	C. tropicalis	≤0.125	–	>0.125	≤0.12	–	0.25–0.50	≥1
Posaconazole	*C. albicans*	≤0.06	–	>0.06	NEY	NEY	NEY	NEY
	C. glabrata	IE	IE	IE	NEY	NEY	NEY	NEY
	C. krusei	IE	IE	IE	NEY	NEY	NEY	NEY
	C. parapsilosis	≤0.06	–	>0.06	NEY	NEY	NEY	NEY
	C. tropicalis	≤0.06	–	>0.06	NEY	NEY	NEY	NEY
Caspofungin	*C. albicans*	NEY	NEY	NEY	≤0.25	–	0.50	≥1
	C. glabrata	NEY	NEY	NEY	≤0.12	–	0.25	≥0.50
	C. krusei	NEY	NEY	NEY	≤0.25	–	0.50	≥1
	C. parapsilosis	NEY	NEY	NEY	≤2	–	4	≥8
	C. tropicalis	NEY	NEY	NEY	≤0.25	–	0.50	≥1
Micafungin	*C. albicans*	NEY	NEY	NEY	≤0.25	–	0.50	≥1
	C. glabrata	NEY	NEY	NEY	≤0.06	–	0.12	≥0.25
	C. krusei	NEY	NEY	NEY	≤0.25	–	0.50	≥1
	C. parapsilosis	NEY	NEY	NEY	≤2	–	4	≥8
	C. tropicalis	NEY	NEY	NEY	≤0.25	–	0.50	≥1
Anidulafungin	*C. albicans*	≤0.03	–	>0.03	≤0.25	–	0.50	≥1
	C. glabrata	≤0.06	–	>0.06	≤0.12	–	0.25	≥0.50
	C. krusei	≤0.06	–	>0.06	≤0.25	–	0.50	≥1
	C. parapsilosis	PT	PT	PT	≤2	–	4	≥8
	C. tropicalis	≤0.06	–	>0.06	≤0.25	–	0.50	≥1

NEY, breakpoints have not been established yet; IE, insufficient evidence to set breakpoints; PT, susceptibility testing not recommended as the species is a poor target for therapy with the drug; S-DD, susceptible dependant on dose.
Data in mg/L.

Transparency Declarations

M.C.E. has received in the past 5 years grant support from Astellas Pharma, bioMerieux, Gilead Sciences, Merck Sharp and Dohme, Pfizer, Schering-Plough, Soria Melguizo SA, Ferrer International, the European Union, the ALBAN program, the Spanish Agency for International Cooperation, the Spanish Ministry of Culture and Education, The Spanish Health Research Fund, The Instituto de Salud Carlos III, The Ramon Areces Foundation, The Mutua Madrileña Foundation. He has been an advisor/consultant to the Panamerican Health Organization, Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer, and Schering-Plough. He has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering-Plough.

P.E.V. has received research grants from Pfizer, Astellas, Cephalon, Gilead Sciences, Merck and Schering-Plough. He is also a board member and consultant for Pfizer, MSD International, Astellas and Gilead. He has also been paid for development of educational presentations by Nadirex Internation.

M.C.A. has received grant support from Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering-Plough. She has been a consultant or at the advisory board for Gilead Sciences, Merck Sharp and Dohme, Pfizer, Pcovery, and Schering-Plough. She has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering-Plough.

S.A.A. has received investigator initiated research grant support from Pfizer and speaker honoraria from Merck and Pfizer. She has been at the Advisory Board for Pfizer-Turkey.

J.B. has nothing to declare.

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H.E.J. has nothing to declare.

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M.D.R. has received grants, speakers honoraria and travel support from Pfizer, Astellas, MSD and Gilead Sciences. He has also received book royalties from Blackwell Publishing and conference support from Astellas Pharma.

M.A. received, during the past 5 years, research grants and honoraria for talks and consultancy and is a board member for Merck, Pfizer and Gilead.

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E.C. has participated as invited speaker to symposia organized by Gilead, Pfizer, Astellas, Merck, Novartis, and he has been member of advisory boards for Astellas, Pfizer.

O.A.C. is supported by the German Federal Ministry of Research and Education (BMBF grant 01KN1106) and has received research grants from, is an advisor to, or received lecture honoraria from 3M, Actelion, Astellas, Basilea, Bayer, Biocryst, Celgene, Cubist, F2G, Genzyme, Gilead, GSK, Merck/Schering, Miltenyi, Optimer, Pfizer, Quintiles, and Viropharma.

J.G. has nothing to declare.

A.H.G. has received research support from Gilead, Merck, and Sharp & Dohme, Schering. He has acted as speaker and/or consultant for Astellas, Cephalon, Gilead, Merck, Pfizer, Sharp & Dohme, Zeneus/Cephalon, Schering and Vicuron.

R.H. has been a consultant or at the advisory board for Astellas pharma, Basilea, Gilead Sciences, Merck Sharp and Dohme, Novartis, Pfizer and Schering-Plough. He has been paid for talks on behalf of Astellas, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering-Plough. He has also received research grants and investigator fees for a clinical trial from Pfizer.

W.W.H. has received grant support from National Institute of Health Research (NIHR), Medical Research

Council, National Institute for the Replacement, Refinement and Reduction, of Animals in Research, Pfizer, Gilead, Schering-Plough, Merck and Astellas and has served as a consultant for Pfizer, Astellas, Gilead, F2G, Vectura and Schering-Plough. His travel costs to meetings have also been paid by ESCMID.

B.J.K. has received research grants from Bio-Mérieux and Cephalon. He is a consultant to Pfizer and is a member of the Gilead, MSD and Pfizer speaker bureaus.

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W.M. has received grant support from MSD and Pfizer. He had been an advisor to MSD and Pfizer. He has received honoraria for presentations on behalf of MSD/Schering-Plough, and Pfizer.

G.P. has received research grants from Gilead, Pfizer, Astra Zeneca, Novartis, Astellas, GSK and MSD, has acted as paid consultant to Janssen Cilag, Gilead, Astellas, and MSD and is a member of the Gilead, Astellas and MSD speaker' s bureaus. His travel costs have also been covered by ESCMID, Gilead, Astellas, Pfizer.

E.R. has received research support from Pfizer, Gilead, Enzon, Schering Merck, and he has made contributions in advisory boards of Gilead, Astellas, Pfizer. He has also received speaker's fees from Gilead, Cephalon, Pfizer, Wyeth, Schering, Merck, Aventis and Astellas. He has also consulted for Schering, Gilead, Astellas, Pfizer and Merck.

C.V. received grants as speaker/moderator in meetings sponsored by Pfizer, Gilead, MSD, Astellas, Abbott, BMS and received grants for participation in advisory boards by Gilead, Astellas, MSD, Pfizer. Further, he obtained research grants for his institution from Pfizer, MSD, Gilead, Abbott, Jansen, BMS, Novartis. He is member of the SAG (Scientific Advisory Group) for antibacterials and antifungals of CHMP-EMA and consultant for Italian Medical Drug Agency Member of various levels of local Infection Control, Antibiotic Stewardship, Vaccine and HIV Committees (Genoa, Liguria, Italy). A.J.U. has received research grants from MSD (Schering-Plough) and is/was an advisor or received lecture honorarium from Astellas, Aicuris, Basilea, Gilead, MSD and Pfizer.

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