ESCMID* guideline for the diagnosis and management of *Candida* diseases 2012: developing European guidelines in clinical microbiology and infectious diseases

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Abstract

The process to develop a guideline in a European setting remains a challenge. The ESCMID Fungal Infection Study Group (EFISG) successfully achieved this endeavour. After two face-to-face meetings, numerous telephone conferences, and email correspondence, an ESCMID task force (basically composed of members of the Society's Fungal Infection Study Group, EFISG) finalized the ESCMID diagnostic and management/therapeutic guideline for *Candida* diseases. By appreciating various patient populations at risk for *Candida* diseases, four subgroups were predefined, mainly ICU patients, paediatric, HIV/AIDS and patients with malignancies including haematopoietic stem cell transplantation. Besides treatment recommendations, the ESCMID guidelines provide guidance for diagnostic procedures. For the guidelines, questions were formulated to phrase the intention of a given recommendation, for example, outcome. The recommendation was the clinical intervention, which was graded by a score of A–D for the 'Strength of a recommendation'. The 'level of evidence' received a score of I–III. The author panel was approved by ESCMID, European Organisation for Research and Treatment of Cancer, European Group for Blood and Marrow Transplantation, European Society of Intensive Care Medicine and the European Confederation of Medical Mycology. The guidelines followed the framework of GRADE and Appraisal of Guidelines, Research, and Evaluation. The drafted guideline was presented at ECCMID 2011 and points of discussion occurring during that meeting were incorporated into the manuscripts. These ESCMID guidelines for the diagnosis and management of *Candida* diseases provide guidance for clinicians in their daily decision-making process.

Keywords: *Candida*, Europe, framework, guideline development, recommendation
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Introduction

Preparing guidelines in this day and age can be likened to the quest of the search for the Holy Grail. Numerous guidelines have been published in a variety of countries and by different scientific societies. All have the common goal of proving clinicians with best guidance for their daily working environment. Obviously, there is no single pathway to the truth in the field of medicine because science and the art of medicine are in a constant state of flux, published data might have already become obsolete and its interpretation might be biased unwittingly.

Nevertheless, it was apparent that certain guidelines for Europe are missing. Firstly, the majority of guidelines focus on treatment, usually only one host group at risk, and to a far lesser extent only a few focus on diagnostic procedures [1–10]. Moreover, North American guidelines are frequently cited in the literature, and this demonstrates their clear dominance [11–15]. Hence, recommendations for diagnostic procedures provided a clear impetus to our group of microbiologists, pathologists, haematologists and infectious diseases physicians (some with dual or more qualifications). In addition, differences in epidemiology by geography, age and local factors needed some attention. Our aim was to provide comprehensive European guidelines focusing on a single fungal disease entity caused by a single genus, namely *Candida* species to allow comprehensive coverage of diagnostics and treatment, recognizing that not all patient risk are alike. It became obvious very quickly that a matrix was needed to cover all topics of interest. This needed to be considered during the guidelines preparation. The guidelines are published as a supplement to CMI and aim to provide greater awareness and better insights into *Candida* diseases for the clinicians.

It was decided that the guidelines for the diagnosis and management of *Candida* diseases is divided into five separate parts, each of which can be used as stand-alone recommendations of the ESCMID treatment management guideline for each risk group of patients and diagnostic procedures.

Methods

Author panel recruitment and organization

The development of any guideline requires certain steps to ensure the production of an unbiased, independent and high-quality document. The executive board of EFISG decided to proceed first with a guideline for *Candida* diseases. The members of the EFISG group were first asked if they wanted to participate. Participants were chosen on the basis of their expertise in the field of medical mycology and in particular *Candida* disease, and further had experience in generating guidelines (Fig. 1). Contact was made through the ESCMID Executive Committee with four different European scientific societies. European Group for Blood and Marrow Transplantation (EBMT), European Confederation of Medical Mycology (ECMM), European Organisation for Research and Treatment of Cancer (EORTC) and European Society of Intensive Care Medicine (ESICM) approved the list of experts and made additional suggestions for experts. Some of the nominees are also members of the ESCMID and were included into the group as panel authors. Experts who were not

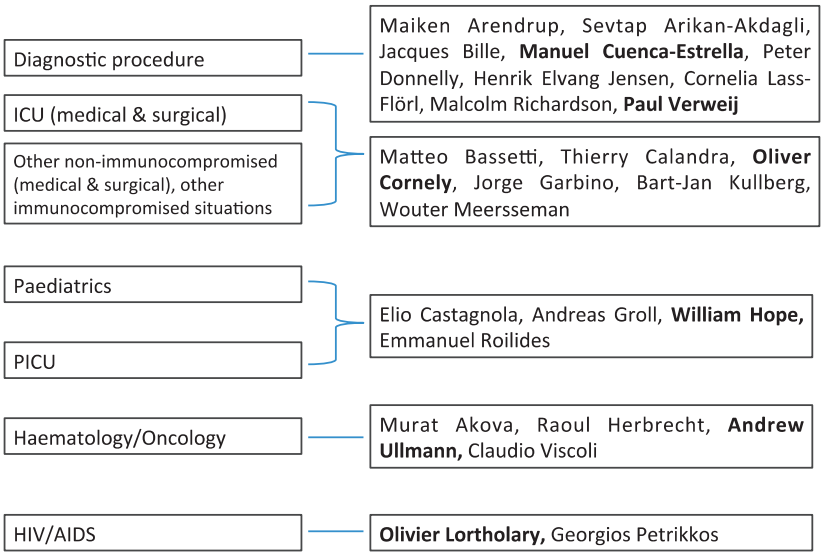

FIG. 1. Working modules and experts participating in the development of the guidelines (susceptibility testing is included for the diagnostic procedures).

selected were asked to peer review the guideline to ensure further quality, although the final decision for the choice of peer reviewers rested with the Editor-in-Chief of CMI. These expert reviewers from the European scientific societies are acknowledged in this paper. This is a novel procedure because reviewers are usually not explicitly mentioned in terms of which papers they have reviewed.

Obviously, to achieve its aim, to provide a European guideline, the group needed to balance between different geographical regions of Europe. The list of representatives of the various European countries is provided in Table 1. For further proficiency, a group coordinator of each subgroup was nominated to provide and present the results of the discussion of this subgroup to the plenary sessions. The subgroups were set up by EFISG. They searched for relevant literature (by PubMed). This literature database was made available to the whole panel on an ftp server of ESCMID. During 2010–2012, documents and views were shared by email, teleconferences and face-to-face meetings. Once a first consensus was reached, the preliminary recommendations were presented to the whole group, that is, the other authors, and subject to wide discussion, developed further, and finalized as a group consensus. Two weekend meetings took place in 2010 and 2011 to finalize the guidelines. The finished guidelines were presented during a workshop session at the ECCMID 2011, and points of discussion occurring during that meeting were incorporated into the final publicized manuscripts. The organization plan used for the guideline is provided in Fig. 2.

TABLE 1. List of the representatives associated with the country

Country	Number (ID)	Number (CM and diagnostic experts)	Total number
Austria	0	1	1
Belgium	1	0	1
Denmark	0	1 + 1[a]	2
France	1 + 1[b]	0	2
Germany	3[c]	0	3
Greece	2	0	2
Italy	3	0	3
Netherlands	1	2	3
Spain	0	1	1
Switzerland	2	1[d]	3
Turkey	1	1[d]	2
United Kingdom	1	1	2

ID, infectious diseases specialist; CM, clinical microbiologist.
[a]Pathologist.
[b]Haematologist.
[c]Dual trained in ID and haematology.
[d]Dual trained in ID and CM.

Intention of the recommendation with defined intervention

During the preparation process, new ideas were incorporated to provide best clinical guidance. Pragmatic questions arising in everyday patient care needed to be addressed appropriately. For this reason, the 'intention' for a recommendation was defined beforehand and framed in terms of 'What does the clinician want?' and a response was tailored to address the different aspects of a given *Candida* disease. Obviously, the diagnostic and therapeutic intervention that

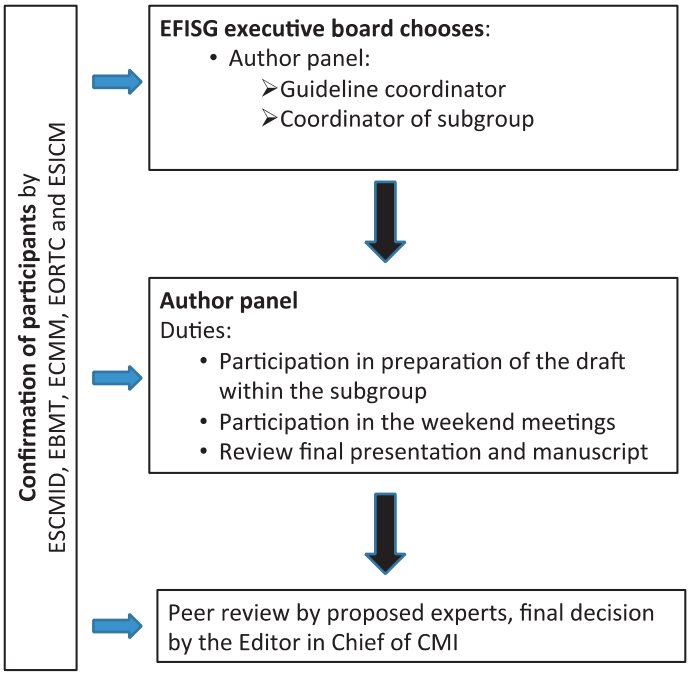

FIG. 2. Organization plan of the guidelines.

had the greatest impact on survival of the patient was given the highest priority in terms of a recommendation.

Certain recommendations were originally controversial. Guidelines are no consensus meeting, but nevertheless, a majority vote was a necessity to formulate a recommendation if a major disagreement occurred. Only a few of the discussions were intense but only had one common goal in mind—to provide the best option for diagnosis and therapy. But whatever the decision, it was one we ensured to be the best for patients.

Every recommendation within the guidelines attempts to indicate clearly the intention (e.g. improved survival) and to describe the diagnostic or therapeutic option (intervention). Therefore, the guidelines follow the principles of the 'Grades of Recommendations, Assessment, Development, and Evaluation' (GRADE) [16]. For every recommendation, the following three questions were considered:

1. What do clinicians want (outcomes)? What is their intention?
2. Which option is better for patients? What intervention is needed to reach the desired outcome?
3. Review the chosen option whether it is truly better or not by adequate review of the literature.

These guidelines also adopted the 'Appraisal of Guidelines, Research and Evaluation' (AGREE) items for the development of guidelines as well [17,18] and basically all domains of AGREE were addressed:

1. Scope and purpose, for example, clinical questions covered by the guideline is described.
2. Stakeholder involvement, for example, the patient's view and preferences have been sought.
3. Rigours of development, for example, the health-related benefits, side effects and risks have been considered in formulating the recommendations.
4. Clarity of presentation, for example, key recommendations are easily identifiable, i.e. tables.
5. Applications, for example, the potential cost-related implications of applying the recommendations have been considered.
6. Editorial independence, for example, the guideline is editorially independent from the funding body.

Within the guideline, questions were formulated and answered according to their clinical importance. Because the guideline author panel appreciated that not all patients were alike, various risk groups were defined according to risk and handled accordingly, that is, patients with HIV/AIDS, those in the ICU, transplant recipients, haematological malignancies and cancer and paediatric populations. At all times, the patient's view and preferences were kept to the fore. One good example that caused some heated debates was the recommendation of not administrating amphotericin B deoxycholate to adults. This drug formulation with considerable toxicity, morbidity and mortality issues, but in regard to acquisition costs relatively cheap has better alternatives at least in Europe available albeit at greater costs. The responsibility to ensure good medical help needed to be considered, and the follow-up costs for the numerous side effects would make the choice of a less cheaper drug acceptable [19]. The ethical dilemma although is obvious but on balance, it was felt that given the facts, the choice of a more expensive formulation was acceptable.

Strength of recommendation

Numerous grading systems of recommendations exist, and it is imperative that they should be not too complicated to understand for the user. Hence, we utilized a similar system as previously employed by the Canadian Task Force of the Periodic Health Examination and the IDSA [12,20]. This is a four-category grading system for the 'strength of a recommendation'. Two extreme ends of the grading system were important: (A) ESCMID strongly supports a recommendation for use and on the other side: (D) ESCMID recommends against the use. This differentiation was important to clearly define treatment management for or against the use of a given interventions. The grade C is weighted with the evidence available and could be considered optional (Table 2). The grading of the 'strength of a recommendation' can be compared to traffic lights, with green indicating the recommendation for use and red the recommendation against use.

The 'strength of a recommendation' cannot easily be applied to diagnostic recommendations. Therefore, an alter-

TABLE 2. Strength of the ESCMID recommendation and quality of evidence

Strength of a recommendation	
Grade A	ESCMID strongly supports a recommendation for use
Grade B	ESCMID moderately supports a recommendation for use
Grade C	ESCMID marginally supports a recommendation for use
Grade D	ESCMID supports a recommendation against use
Quality of evidence	
Level I	Evidence from at least one properly designed randomized controlled trial
Level II*	Evidence from at least one well-designed clinical trial, without randomization; from cohort or case–controlled analytic studies (preferably from >1 centre); from multiple time series; or from dramatic results of uncontrolled experiments
Level III	Evidence from opinions of respected authorities, based on clinical experience, descriptive case studies

*Added index:
$_r$: Meta-analysis or systematic review of randomized controlled trials.
$_t$: Transferred evidence, that is, results from different patients' cohorts, or similar immune-status situation.
$_h$: Comparator group is a historical control.
$_u$: Uncontrolled trial.
$_a$: Published abstract (presented at an international symposium or meeting).

TABLE 3. System used in these guidelines for grading quality of evidence about the accuracy of biomarker detection procedures in the diagnosis of candidiasis

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 (1) at least one well-designed prospective single-centre cross-sectional or cohort study or (2) a properly designed retrospective multicentre cross-sectional or cohort study or (3) 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).

native system was adopted for biomarkers (non-cultural techniques), which included test accuracy, as this plays a pivotal role in providing an appropriate diagnosis. The GRADE system was used to grade the 'strength of a recommendation' and 'quality of evidence' [21,22]. Therefore, the system was slightly modified and is applicable for biomarkers (non-cultural techniques) only. The term accuracy of a test was introduced, and a grading system was implemented on those calculated numbers (Table 3). The grading system used a clear statement, that is, highly recommended, recommended and not recommended and did not utilize the alphabet system for treatment. If no published data were available to support any kind of recommendation, no recommendation for the test was provided. The equation for accuracy was the sum of true positive and true negative tests divided by the sum of all tests performed. The wording for the 'quality of evidence' was changed only marginally to maintain a streamlined recommendation grading system (Table 3).

Quality of evidence

The 'strength of a recommendation' was largely based on the available studies and publications. Although there were obvious exceptions, for example, drawing blood cultures for candidaemia because in this case, no literature was cited. On the other hand, various publications discussed issues surrounding the selection of appropriate literature [23,24]. This literature should support the judgement made by the panel. This guideline is not a classical systematic review of the literature. It was clearly intended to review the literature on the impact of the test and alternative management strategies on the outcome in patients [25]. The panel reviewed the available evidence and recognized its limitations but interpretation bias cannot be ruled out entirely. The panel always kept its focus on the need for an evidence-based (medicine) justification. Despite some limitations in the selection process, by which means every subgroup was internally responsible for, all retrieved literature (by PubMed) were considered. A meta-analysis was not intended and not all retrieved literature was cited. Nevertheless, we rated the evidence as the Canadian Task Force on the Periodic Health Examination and the IDSA [12,20]. One modification was added to the level II of 'Quality of Evidence'. The panel recognized that not all questions could be answered by published literature but, for example, similar immunological situations or a substantial abstract from larger international recognized scientific meetings could be used as 'evidence'. Therefore, especially for academic purposes and to increase transparency, indices were added to the level II of 'Quality of Evidence' (Table 1).

Discussion and conclusions

These ESCMID guidelines provide a European-wide guideline for clinical guidance in the diagnosis and treatment of *Candida* diseases. The guidelines offer besides diagnostic also treatment recommendations for various patients' groups and are weighted differently according to available literature. The basis of these guidelines were to follow the framework provided by GRADE and AGREE [16–18,24–26]. The panel fully acknowledges numerous published guidelines and recognized some shortcomings that the ESCMID guideline tried to overcome: Mainly providing an independent European guideline for diagnostic procedures and treatment recommendations suitable for all patients at risk for *Candida* diseases. Obviously, not all patient profiles are homogeneous, as their risk profile and response to therapy may differ. Minor changes in the view of rating systems were implemented into this guideline.

These guideline should also serve as a tool for guiding the clinical care of patients in Europe. The ESCMID guidelines consist of text but also includes tables that are easily readable. The development of the guidelines was made transparent, and the panel was also supported by other European societies as well as a broad panel of experts from various backgrounds and countries. The guidelines were (peer-) reviewed by other experts in the field of medical mycology and who were in part suggested by other European societies. Their pivotal role by peer review in the process of the guideline development cannot be underestimated and the entire panel expresses their gratitude by acknowledging their work at the end of this manuscript.

The development of guidelines comes with a price tag, as there are inevitably costs incurred by travel and accommodation. Funding was neither sought nor granted by biomedical or pharmaceutical companies for the development of these guidelines. Additionally, biomedical or pharmaceutical companies were not involved in the development of these guidelines neither as observers or discussants. For this reason, we received a grant of 50 000€ from ESCMID to accomplish this task. Transparency declarations of the panel are provided to every guideline. This support by ESCMID guaranteed independence including editorial independence.

Challenges remain for the guidelines. Trying to assess *Candida* epidemiology in Europe remained a challenge because only a few adequate European publications were available. The guidelines want to serve as a tool for guidance as for local (hospital) guidelines, which would require individual adaptations to meet local needs [27]. Therefore, it remains important to have European guidelines that can be adapted to local use.

Costs incurred by diagnostic procedures or treatments are not considered mainly because of the differences of reimbursement systems in Europe. Cost effectiveness calculations of different treatment modalities have been assessed by others but are only applicable for the specific countries (e.g. [28]).

Obviously, more research is needed in the field of *Candida* diseases particular in epidemiology and the development of resistance. 'Strength of a recommendation' with a grading of 'C' highlights our obligation to further work in this area to arrive at a more adequate or satisfactory answer. The EFISG is actively developing guidelines in other fields of medical mycology (e.g. rare and emerging fungi and aspergillosis) and will seek cooperation with other scientific societies sharing this goal. The current *Candida* guidelines are planned to be reviewed in the next 5 years to ensure it remains up to date. If new and pivotal clinical data become available, then the planned update will take place earlier.

In summary, these ESCMID guidelines are independent of any industry funding or support or influence and were drafted as an independent recommendation by 25 European experts from 12 countries. The panel of authors hopes that these ESCMID guidelines for the diagnosis and management of *Candida* diseases will provide adequate guidance for clinicians in everyday decision-making process, which can be easily adapted to their clinical practice.

Transparency Declarations

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.

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, Sanofi Pasteur, Quintiles, Viropharma.

J.P.D. has received grant support from, Astellas, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering Plough. He has been a consultant or on an advisory board for Astellas, Gilead Sciences, Merck Sharp and Dohme, and Pfizer. He has received remuneration for giving lectures on behalf of Gilead Sciences, Merck and Pfizer.

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

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.

M.B. has received research grants from Pfizer, MSD and Astellas and is/was an advisor or received lecture honorarium from Astellas, Aventis, Bayer, Cephalon, Cubist, Gilead, MSD, Novartis, Shionogi, Pfizer, Teva and Vifor.

J.B. has nothing to declare.

T.C. is member of the Speaker bureau, and is advisor or consultant for Astellas, Baxter; bioMérieux, EISAI, Evolva, Novartis, Merck Sharp & Dohme-Chibret AG, Immunexpress, Eli Lilly Suisse, Pfizer. Grant support from Baxter, bioMérieux, Merck Sharp & Dohme-Chibret AG, Roche Diagnostic. He has also received payment from MSD, Institut Pasteur and Gilead Sciences for development of educational presentations, as well as royalties from Elsevier.

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. He also has received payment for development of educational presentations and for lectures and consultancy.

J.G. has nothing to declare.

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

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. His travel and accommodation expenses have also been covered by Pfizer and Gilead and a research grant 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 covered by ESCMID.

H.E.J. has nothing to declare.

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.

C.L.-F. has received grant support in the past 5 years from Astellas Pharma, Gilead Sciences, Pfizer, Schering Plough and Merck Sharp and Dohme. She has been an advisor/consultant to Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering Plough. She has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma and Schering Plough.

O.L. is a member of the MSD board, is a consultant for Astellas and Gilead Sciences, and received grants or speaker's fees from MSD, Astellas, Gilead Sciences and Pfizer.

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, Astra Zeneca, Novartis, Astellas, GSK, Pfizer 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.

M.D.R. has received grants, speaker's honoraria and travel support from Pfizer, Astellas, ESCMID, MSD and Gilead Sciences. He has also received book royalties from Blackwell Publishing.

E.R. has received research support from Pfizer, Enzon, Gilead, Merck and he has made contributions in advisory boards of Gilead, Astellas, Pfizer. He has also been a consultant/speaker for Schering, Gilead, Astellas, Pfizer, Merck, Wyeth, Cephalon and Aventis.

P.E.V. has received research grants from Pfizer, Astellas, Cephalon, Gilead Sciences, Merck and Schering-Plough.

C.V. received grants as speaker/moderator in meetings sponsored by Pfizer, Gilead, MSD, Astellas, Abbott, Nadirex International, 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).

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.

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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.

J.P.D. has received grant support from, Astellas, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering-Plough. He has been a consultant or on an advisory board for Astellas, Gilead Sciences, Merck Sharp and Dohme, and Pfizer. He has received remuneration for giving lectures on behalf of Gilead Sciences, Merck, and Pfizer.

H.E.J. has nothing to declare.

C.L.-F. has received grant support in the past 5 years from Astellas Pharma, Gilead Sciences, Pfizer, Schering-Plough and Merck Sharp and Dohme. She has been an advisor/consultant to Astellas Pharma, Gilead Sciences, Merck Sharp and Dohme, Pfizer and Schering-Plough. She has been paid for talks on behalf of Gilead Sciences, Merck Sharp and Dohme, Pfizer, Astellas Pharma, Pfizer and Schering-Plough. Her travel and meeting expenses have also been paid by the above.

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.

M.B. has received research grants from Pfizer, MSD and Astellas and is/was an advisor or received lecture honorarium from Astellas, Angelini Farmaceutici, Astra Zeneca, Aventis, Bayer, Cephalon, Cubist, Gilead, MSD, Novartis, Shionogi, Pfizer, Teva and Vifor. He is also a board member of Pfizer, Angelini Farmaceutici, Cubist, MSD, Astellas, Novartis, Astra Zeneca.

T.C. is member of the Speaker bureau and is advisor or consultant for Astellas, Baxter; bioMérieux, EISAI, Evolva, Eli Lilly Suisse, Novartis, Merck Sharp & Dohme-Chibret AG, Pfizer. Grant support from Baxter, bioMérieux, Merck Sharp and Dohme-Chibret AG, Roche Diagnostic. He has also received payment for educational presentations from MSD, Institut Pasteur and Gilead Sciences.

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.

O.L. is a member of the MSD board, is a consultant for Astellas and Gilead Sciences and received grants or speaker's fees from MSD, Astellas, Gilead Sciences and Pfizer.

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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