

# METHODS IN MOLECULAR BIOLOGY

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# Human Fungal Pathogen Identification

## Methods and Protocols

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## Preface

Fungal infections constitute an ever-growing healthcare problem worldwide. Invasive fungal disease (IFD) is a leading cause of morbidity and mortality in severely immunocompromised individuals, including a variety of critically ill patients. High-risk conditions for IFD include hematologic malignancies, hematopoietic stem cell or solid organ transplantations, primary or acquired immunodeficiencies such as AIDS, long-term treatment at intensive care units, preterm birth, long-term immunosuppressive therapy, broad-spectrum antibiotic therapy, and long presence of central indwelling catheters. Despite the availability of a number of potent antifungal drugs, successful treatment of IFD is often hampered by the limited diagnostic options which neither permit rapid and reliable identification of systemic, acute, or latent fungal infections nor facilitate assessment of host susceptibility. The resulting delay in the onset of antifungal therapy is an important factor contributing to the poor overall clinical outcome of IFD. Moreover, the widespread use of prophylactic or empirical treatment without firm evidence of IFD leads to a high rate of overtreatment associated with considerable toxic side effects, and broad-spectrum antimycotic therapy administered against unidentified fungal pathogens can promote the evolution of clinical drug resistance.

The vast majority of IFDs are caused by *Candida* and *Aspergillus* species. However, changes in the epidemiology have occurred over the last decades, with a number of newly emerging fungal pathogens affecting even immunocompetent patients. Additionally, rare but pronounced antifungal drug resistance of some major fungal pathogens (e.g., *C. glabrata*, *C. krusei*, *A. terreus*) poses a serious challenge for treatment. Despite current evidence that the innate immune surveillance is critical for defence against invasive fungal infections, host predisposition to IFD, which would permit targeted preventive treatment, is barely considered in diagnostic procedures. Microbiological diagnostic criteria of IFD provided by the EORTC/MSG (European Organization for Research and Treatment of Cancer/Mycoses Study Group) include the detection of fungal pathogens by cytology, direct microscopy, or culture and serological detection of antigens or cell wall constituents, such as galactomannan or  $\beta$ -d-glucan. By contrast, molecular detection of fungal nucleic acids is currently not accepted as microbiological evidence of IFD due to insufficient clinical evidence and lack of standardized and validated assays.

Rapid, reliable, and species-specific diagnosis of fungal pathogens causing IFD is a prerequisite for cost-effective and successful therapy but remains one of the great challenges. The changing epidemiology, the increasing variety of fungal pathogens, and the rising number of affected patients create a growing demand for broad-range and cost-effective clinical diagnostic tests, also permitting the prediction of pathogenicity and resistance to antifungal agents. The knowledge of host-related predisposing factors and stratified treatment options facilitating timely onset of adequate antifungal therapy are critical for successful clinical management and outcome of IFD. This requires not only rapid diagnosis of a fungal infection and identification of the causative species but also

assessment of pathogen/host factors related to pathogenicity, susceptibility, and response to treatment. The present book responds to the great need for timely and authoritative information offering a comprehensive overview of the current state of the art in fungal diagnostics. Moreover, it addresses ongoing developments expected to provide a basis for targeted treatment strategies resulting in improved outcome of invasive mycoses.

*Vienna, Austria*

*Thomas Lion*

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# Contents

<i>Preface</i> . . . . .	v
<i>Contributors</i> . . . . .	ix

## PART I INTRODUCTORY CHAPTERS

1	Current Challenges in the Diagnosis of Fungal Infections.....	3
	<i>Cornelia Lass-Flörl</i>	
2	The Changing Epidemiology of Invasive Fungal Infections.....	17
	<i>David A. Enoch, Huina Yang, Sani H. Aliyu, and Christianne Micallef</i>	
3	Current Algorithms in Fungal Diagnosis in the Immunocompromised Host.....	67
	<i>Thomas Lehrnbecher, Karsten Becker, and Andreas H. Groll</i>	
4	Commercial Molecular Tests for Fungal Diagnosis from a Practical Point of View.....	85
	<i>Michaela Lackner and Cornelia Lass-Flörl</i>	
5	Systemic Antifungal Agents: Current Status and Projected Future Developments.....	107
	<i>Seyedmojtaba Seyedmousavi, Haleh Rafati, Macit Ilkit, Ali Toloee, Mohammad T. Hedayati, and Paul Verweij</i>	
6	Fungal-Grade Reagents and Materials for Molecular Analysis.....	141
	<i>Michael G. Lorenz, Michael Lustig, and Marina Linow</i>	

## PART II HOST SUSCEPTIBILITY AND DEFENSE

7	Host-Derived Biomarkers for Risk Assessment of Invasive Fungal Diseases .....	153
	<i>Cristina Cunha, Samuel M. Gonçalves, and Agostinho Carvalho</i>	
8	Assessment of Immune Responses to Fungal Infections: Identification and Characterization of Immune Cells in the Infected Tissue.....	167
	<i>Florian Sparber and Salomé LeibundGut-Landmann</i>	

## PART III SCREENING APPROACHES TO FUNGAL PATHOGEN DETECTION

9	Histopathology .....	185
	<i>Renate Kain</i>	
10	Culture-Based Techniques.....	195
	<i>Birgit Willinger</i>	
11	Serological Approaches .....	209
	<i>Barbora Weinbergerova, Iva Kocmanova, Zdenek Racil, and Jiri Mayer</i>	
12	Isolation of Nucleic Acids for Fungal Diagnosis.....	223
	<i>P. Lewis White and Rosemary A. Barnes</i>	

13	Prerequisites for Control of Contamination in Fungal Diagnosis .....	249
	<i>Stefan Czurda and Thomas Lion</i>	
14	Broad-Spectrum Molecular Detection of Fungal Nucleic Acids by PCR-Based Amplification Techniques .....	257
	<i>Stefan Czurda and Thomas Lion</i>	
15	Genus- and Species-Specific PCR Detection Methods.....	267
	<i>Jan Springer and Jürgen Löffler</i>	
16	Identification of Fungal Pathogens in Tissue Samples from Patients with Proven Invasive Infection by Fluorescence <i>In Situ</i> Hybridization .....	281
	<i>Ilka McCormick Smith and Volker Rickerts</i>	
17	Nuclear Magnetic Resonance Spectroscopy-Based Identification of Yeast .....	289
	<i>Uwe Himmelreich, Tania C. Sorrell, and Heide-Marie Daniel</i>	
18	T2 Magnetic Resonance for Fungal Diagnosis .....	305
	<i>Fainareti N. Zervou, Ioannis M. Zacharioudakis, Jaclynn Kurpewski, and Eleftherios Mylonakis</i>	
 PART IV IDENTIFICATION OF FUNGAL SPECIES		
19	Fungal Species Identification by MALDI-ToF Mass Spectrometry .....	323
	<i>Oliver Bader</i>	
20	Immunological Identification of Fungal Species.....	339
	<i>Filomena Nogueira, Fabian Istel, Leonel Pereira, Michael Tscherner, and Karl Kuchler</i>	
21	The Molecular Blueprint of a Fungus by Next-Generation Sequencing (NGS)....	361
	<i>Christian Grumaz, Philipp Kirstahler, and Kai Sohn</i>	
22	Microarray Technologies in Fungal Diagnostics .....	385
	<i>Steffen Rupp</i>	
 PART V DRUG RESISTANCE TESTING		
23	Molecular Detection of Resistance to Echinocandins .....	413
	<i>Brunella Posteraro, Antonietta Vella, Elena De Carolis, and Maurizio Sanguinetti</i>	
24	Molecular Detection of Resistance to Azole Components .....	423
	<i>Brunella Posteraro, Antonietta Vella, Elena De Carolis, and Maurizio Sanguinetti</i>	
 PART VI NOVEL EXPERIMENTAL APPROACHES		
25	Immune Cell-Supplemented Human Skin Model for Studying Fungal Infections .....	439
	<i>Andreas Kühbacher, Kai Sohn, Anke Burger-Kentischer, and Steffen Rupp</i>	
	<i>Index</i> .....	451

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