Dual Panel Multiplex PCR Assay for Rapid Detection of Medically Important Fungi and Resistant Species of Candida and Aspergillus

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Abstract

Invasive fungal infections (IFIs) have risen dramatically in recent years among high risk immunocompromised patients. Rapid detection of fungal pathogens is crucial to timely and accurate antifungal therapy. Two multiplex polymerase chain reaction (PCR) assays were developed to detect major fungal species that cause invasive infections and identify resistant species. Genus specific primers for Candida, Aspergillus, Fusarium and species specific primers for Candida glabrata, Candida krusei and Aspergillus terreus which are known to be clinically resistant species, were designed from the internal transcribed spacer (ITS) regions of ribosomal ribonucleic acid (rRNA) gene complex. Both assays were performed simultaneously to promote rapid detection of fungal isolates based on distinct amplicon sizes. Inclusion of the universal fungal primers ITS 1 and ITS 4 in the genus specific assay produced a second amplicon for each isolate which served to confirm the detection of a fungal target. The limit of detection for the genus specific assay was 1 nanogram (ng) deoxyribonucleic acid (DNA) for Aspergillus fumigatus and Candida albicans, 0.1 ng DNA for Fusarium solani, while the species-specific assay detected 0.1 ng DNA of A. terreus and 10 picogram (pg) DNA of C. krusei and C. glabrata. The multiplex PCR assays, apart from universal detection of any fungal target, are able to detect clinically important fungi and differentiate resistant species rapidly and accurately, which can contribute to timely implementation of effective antifungal regime.

Keywords

Author Keywords: Aspergillus; Candida; detection; Fusarium; multiplex PCR

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Cited References: 39

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1. **Candida krusei fungemia - An escalating serious infection in immunocompromised patients** — Times Cited: 90
By: Abbas, J; Bodey, GP; Hanna, HA; et al.
ARCHIVES OF INTERNAL MEDICINE Volume: 160 Issue: 17 Pages: 2659-2664 Published: SEP 25 2000

2. **Antifungal susceptibility profile of clinical Fusarium spp. isolates identified by molecular methods** — Times Cited: 127
By: Alastruey-Izquierdo, Ana; Cuenca-Estrella, Manuel; Monzon, Araceli; et al.
JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY Volume: 61 Issue: 4 Pages: 805-809 Published: APR 2008

3. **A multiplex PCR method for detection of Aspergillus spp. and Mycobacterium tuberculosis in BAL specimens** — Times Cited: 1
By: Amini, F; Kachuei, R.; Noorbakhsh, F; et al.
Journal of Medical Mycology Volume: 25 Issue: 2 Pages: 59-64 Published: 2015
[Show additional data]

4. **PCR Diagnosis of Invasive Candidiasis: Systematic Review and Meta-Analysis** — Times Cited: 192
By: Avni, Tomer; Leibovici, Leonard; Paul, Mical
JOURNAL OF CLINICAL MICROBIOLOGY Volume: 49 Issue: 2 Pages: 665-670 Published: FEB 2011

5. **Rapid diagnosis of invasive fungal infections.** — Times Cited: 1
By: Azab, M. M.; Taleb, A. F. A.; Mohamed, N. A. E; et al.
International Journal Current Microbiology and Applied Sciences Volume: 4 Issue: 11 Pages: 470-486 Published: 2015
[Show additional data]

6. **Laboratory diagnostics of invasive fungal infections: an overview with emphasis on molecular approach** — Times Cited: 11
By: Baskova, Lenka; Buchta, Vladimir
FOLIA MICROBIOLOGICA Volume: 57 Issue: 5 Pages: 421-430 Published: SEP 2012

7. **The Pan-AC assay: a single-reaction real-time PCR test for quantitative detection of a broad range of Aspergillus and Candida species** — Times Cited: 44
By: Baskova, Lenka; Landlinger, Christine; Preuner, Sandra; et al.
JOURNAL OF MEDICAL MICROBIOLOGY Volume: 56 Issue: 9 Pages: 1167-1173 Published: SEP 2007

8. **Strain variation among and antifungal susceptibilities of isolates of Candida krusei** — Times Cited: 37
By: Berrouane, YF; Hollis, RJ; Pfaller, MA
JOURNAL OF CLINICAL MICROBIOLOGY Volume: 34 Issue: 7 Pages: 1856-1858 Published: JUL 1996

9. **New Insight into Amphotericin B Resistance in Aspergillus terreus** — Times Cited: 36
By: Blum, Gerhard; Hoertnagl, Caroline; Jukic, Emina; et al.
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY Volume: 57 Issue: 4 Pages: 1583-1588 Published: APR 2013

10. **Seminested PCR for detection and identification of Candida species directly from blood culture bottles** — Times Cited: 8
By: Cerikcioglu, Nilguen; Aksu, Burak; Dal, Tuba Demirel; et al.
NEW MICROBIOLOGICA Volume: 33 Issue: 1 Pages: 57-62 Published: JAN 2010

11. **Rapid identification of yeasts in positive blood cultures by a multiplex PCR method** — Times Cited: 77
By: Chang, HC; Leaw, SN; Huang, AH; et al.
JOURNAL OF CLINICAL MICROBIOLOGY Volume: 39 Issue: 10 Pages: 3466-3471 Published: OCT 2001

12. **Update on the epidemiology and diagnosis of invasive fungal infection** — Times Cited: 45
By: Cuenca-Estrella, Manuel; Bernal-Martinez, Leticia; Jose Buitrago, Maria; et al.
INTERNATIONAL JOURNAL OF ANTIMICROBIAL AGENTS Volume: 32 Pages: S143-S147 Published: NOV 2008

13. **Invasive aspergillosis** — Times Cited: 1,248
By: Denning, DW
CLINICAL INFECTIOUS DISEASES Volume: 26 Issue: 4 Pages: 781-803 Published: APR 1998

14. **Fungal infections in the critically ill** — Times Cited: 3
By: Dhillon, Rishi H.; Clark, John
TRENDS IN ANAESTHESIA AND CRITICAL CARE Volume: 1 Issue: 4 Pages: 210-218 Published: AUG 2011

15. **Human fusariosis** — Times Cited: 204
By: Dignani, MC; Anaissie, E
CLINICAL MICROBIOLOGY AND INFECTION Volume: 10 Supplement: 1 Pages: 67-75 Published: MAR 2004

16. **Real-time PCR: A rapid and sensitive method for diagnosis of dermatophyte induced onychomycosis, a comparative study.** — Times Cited: 2
By: Emam, S. M.; Abd El-salam, O. H.

Alexandria Journal of Medicine Volume: 52 Issue: 1 Pages: 1-8 Published: 2015

17. **Emerging systemic fungal infections** — Times Cited: 25
By: Galimberti, Ricardo; Clara Torre, Ana; Carolina Baztan, Maria; et al.
CLINICS IN DERMATOLOGY Volume: 30 Issue: 6 Pages: 633-650 Published: NOV-DEC 2012

18. **Eukaryotic genome size databases** — Times Cited: 245
By: Gregory, T. Ryan; Nicol, James A.; Tamm, Heidi; et al.
NUCLEIC ACIDS RESEARCH Volume: 35 Special Issue: SI Pages: D332-D338 Published: JAN 2007

19. **Identification of Aspergillus species using internal transcribed spacer regions 1 and 2** — Times Cited: 236
By: Henry, T; Iwen, PC; Hinrichs, SH
JOURNAL OF CLINICAL MICROBIOLOGY Volume: 38 Issue: 4 Pages: 1510-1515 Published: APR 2000

20. **A novel, multiplex, real-time PCR-based approach for the detection of the commonly occurring pathogenic fungi and bacteria** — Times Cited: 8
By: Horvath, Adam; Peto, Zoltan; Urban, Edit; et al.
BMC MICROBIOLOGY Volume: 13 Article Number: 300 Published: DEC 23 2013

21. **Species-Specific Identification of a Wide Range of Clinically Relevant Fungal Pathogens by Use of Luminex xMAP Technology** — Times Cited: 58
By: Landlinger, C.; Preuner, S.; Willinger, B.; et al.
JOURNAL OF CLINICAL MICROBIOLOGY Volume: 47 Issue: 4 Pages: 1063-1073 Published: APR 2009

22. **Multiplex PCR for the discrimination of A-fumigatus, A-flavus, A-niger and A-terreus** — Times Cited: 22
By: Logotheti, M.; Kotsovili-Tseleni, A.; Arsenis, G.; et al.
JOURNAL OF MICROBIOLOGICAL METHODS Volume: 76 Issue: 2 Pages: 209-211 Published: FEB 2009

23. **Recent Exposure to Caspofungin or Fluconazole Influences the Epidemiology of Candidemia: a Prospective Multicenter Study Involving 2,441 Patients** — Times Cited: 197
By: Lortholary, Olivier; Desnos-Ollivier, Marie; Sitbon, Karine; et al.
Group Author(s): French Mycosis Study Grp
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY Volume: 55 Issue: 2 Pages: 532-538 Published: FEB 2011

24. **Usefulness of capillary electrophoresis-based multiplex PCR assay for species-specific identification of Candida spp.** — Times Cited: 6
By: Mallus, F.; Martis, S.; Serra, C.; et al.
JOURNAL OF MICROBIOLOGICAL METHODS Volume: 92 Issue: 2 Pages: 150-152 Published: FEB 15 2013

25. **Global population structure of Aspergillus terreus inferred by ISSR typing reveals geographical subclustering** — Times Cited: 22
By: Neal, Carolyn O. S.; Richardson, Aaron O.; Hurst, Steven F.; et al.
BMC MICROBIOLOGY Volume: 11 Article Number: 203 Published: SEP 16 2011

26. **Up to date epidemiology, diagnosis and management of invasive fungal infections** — Times Cited: 25
By: Oren, I.; Paul, M.
CLINICAL MICROBIOLOGY AND INFECTION Volume: 20 Special Issue: SI Supplement: 6 Pages: 1-4 Published: JUN 2014

27. **Epidemiology of invasive candidiasis: a persistent public health problem** — Times Cited: 2,311
By: Pfaller, M. A.; Diekema, D. J.
CLINICAL MICROBIOLOGY REVIEWS Volume: 20 Issue: 1 Pages: 133-+ Published: JAN 2007

28. **Nosocomial Candidiasis: Antifungal Stewardship and the Importance of Rapid Diagnosis** — Times Cited: 61
By: Pfaller, Michael A.; Castanheira, Mariana
MEDICAL MYCOLOGY Volume: 54 Issue: 1 Pages: 1-22 Published: JAN 2016

29. **Towards molecular diagnostics of invasive fungal infections** — Times Cited: 16
By: Preuner, Sandra; Lion, Thomas
EXPERT REVIEW OF MOLECULAR DIAGNOSTICS Volume: 9 Issue: 5 Pages: 397-401 Published: JUL 2009

30. **Changing epidemiology of systemic fungal infections** — Times Cited: 260
By: Richardson, M.; Lass-Floerl, C.
CLINICAL MICROBIOLOGY AND INFECTION Volume: 14 Supplement: 4 Pages: 5-24 Published: MAY 2008

Showing 30 of 39 **View All in Cited References page**