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Performance of Matrix-Assisted Laser Desorption Ionization−Time of Flight Mass Spectrometry for Identification of *Aspergillus*, *Scedosporium*, and *Fusarium* spp. in the Australian Clinical Setting

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We developed an Australian database for the identification of *Aspergillus*, *Scedosporium*, and *Fusarium* species ($n = 28$) by matrix-assisted laser desorption ionization−time of flight mass spectrometry (MALDI-TOF MS). In a challenge against 117 isolates, species identification significantly improved when the in-house-built database was combined with the Bruker Filamentous Fungi Library compared with that for the Bruker library alone (*Aspergillus*, 93% versus 69%; *Fusarium*, 84% versus 42%; and *Scedosporium*, 94% versus 18%, respectively).

Rapid, accurate mold identification is important due to the widening spectrum of pathogens and species-specific differences in antifungal susceptibility (1–3). Matrix-assisted laser desorption ionization−time of flight mass spectrometry (MALDI-TOF MS) has proven useful, but mold identification remains challenged by the limited access to validated purpose-built databases that are necessary because of small species and strain representations in commercial libraries (4–16).

Given the prior poor performance of the Bruker Filamentous Fungi Library v1.0 (Bruker Daltonics, Bremen, Germany) for mold identification using the manufacturer-recommended broth-based protein extraction methods (in our laboratory >50% of isolates were not identified; internal data) and because the geographic generalizability of in-house-built databases is not yet known, we hypothesized that a MS library of molds relevant to our region (17–21) will improve identification. Here, we constructed an in-house database containing 117 strains (see Table S1 in the supplemental material) covering 28 species of *Aspergillus*, *Scedosporium*, and *Fusarium* encountered in Australia. Challenge isolates (also $n = 117$; 21 species) comprising 55 *Aspergillus*, 45 *Fusarium*, and 17 *Scedosporium* clinical strains (Table 1) were then used to assess the performance of the Bruker library alone versus that of the Bruker library supplemented with the in-house library for species identification.

All isolates were identified using phenotypic methods (22) with definitive identification by DNA sequencing of the internal transcribed spacer (ITS) (all isolates), β-tubulin (*Aspergillus* and *Scedosporium* spp.), and partial elongation factor-1alpha (EF-1α) (to identify *Fusarium* to the species complex level) gene regions (23–26). Sequence data were analyzed against the Centraalbureau voor Schimmelkultures (http://www.cbs.knaw.nl/Collections/BioloMICSSequences.aspx?file=all), International Society for Human and Animal Mycology ITS (http://its.mycologylab.org/), and Fusarium-ID (http://www.fusariumdb.org/index.php) databases, and species were assigned using published criteria (27).

Protein extraction for MALDI-TOF MS was performed as previously described (11). The Bruker bacterial test standard (Bruker Daltonics) was used for calibration and *Aspergillus ustus* CBS 261.67T scoring of ≥2.00 was required for quality extraction and spectra acceptability (11). The in-house database was constructed using published protocols (11, 28) with 20 to 25 quality spectra required for mass spectral profile (MSP) creation using default Biotyper settings (Bruker Daltonics).

For challenge isolates, spectra were acquired in technical triplicates using established protocols (11) and queried against (i) the Bruker library and (ii) the Bruker library combined with the in-house database. Manufacturer-recommended cutoff values (for species, log score of ≥2.00; for genus, score of ≥1.70 to ≤1.99) were maintained. Median values and interquartile ranges (IQR) of log scores obtained by the two database sets were calculated for all isolates and then by genera and selected species using SPSS v21 software (SPSS Inc., Chicago, IL). The Wilcoxon signed-rank test was used to compare the median scores and Friedman's two-way analysis of variance to compare the distributions of scores (Fig. 1). McNemar's test was used to compare the frequencies of paired log score data at cutoffs of ≥2.00 versus ≥1.70 and ≥2.00 versus ≥1.80.

Given the importance of protein extraction for acquiring quality spectra, we used a well-validated method (11) to develop our database. All isolate-specific spectra were reproducible and matched well with their corresponding MSP. Comparison of the extraction method used here with another proposed agar-based method (13) for spectra quality may be of clinical interest.

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TABLE 1 Performance according to genera and species of the Bruker library and the Bruker library supplemented with the customized in-house database when evaluated against a set of challenge clinical isolates[a]

Organism (no. isolates)	No. (%) isolates of its genus or species correctly identified by the specified log score value							
	Bruker library alone				Bruker library plus in-house database			
	≥2.00	≥1.70	<1.70 (no ID)	Mis-ID	≥2.00	≥1.70	<1.70 (no ID)	Mis-ID
Aspergillus spp. (55)	38 (69)	43 (78)	8 (14.5)	4 (7.2)	51 (93)	52 (95)	2 (3.6)	1 (1.8)[b]
Aspergillus alliaceus (2)	0 (0)	0 (0)	2 (0)	0 (0)	2 (100)	2 (100)	0 (0)	0 (0)
Aspergillus creber (2)	0 (0)	0 (0)	0 (0)	2 (100)[b]	2 (100)	2 (100)	0 (0)	0 (0)
Aspergillus flavus (5)	5 (100)	5 (100)	0 (0)	0 (0)	5 (100)	5 (100)	0 (0)	0 (0)
Aspergillus fumigatus (14)[c]	14 (100)	14 (100)	0 (0)	0 (0)	14 (100)	14 (100)	0 (0)	0 (0)
Aspergillus lentulus (3)	0 (0)	0 (0)	3 (100)	0 (0)	3 (100)	3 (100)	0 (0)	0 (0)
Aspergillus nidulans (6)	6 (100)	6 (100)	0 (0)	0 (0)	6 (100)	6 (100)	0 (0)	0 (0)
Aspergillus niger (9)	7 (78)	9 (100)	0 (0)	0 (0)	8 (89)	9 (100)	0 (0)	0 (0)
Aspergillus terreus (7)	3 (43)	6 (86)	1 (14.2)	0 (0)	7 (100)	7 (100)	0 (0)	0 (0)
Aspergillus versicolor (3)	3 (100)	3 (100)	0 (0)	0 (0)	3 (100)	3 (100)	0 (0)	0 (0)
Aspergillus viridinutans complex (2)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)
Aspergillus sydowii (2)	0 (0)	0 (0)	0 (0)	2 (100)[b]	1 (50)	1 (50)[b]	0 (0)	1 (50)[b]
Fusarium spp. (45)	19 (42)	38 (84)	7 (15.5)	0 (0)	38 (84)	45 (100)	1 (2.2)	0 (0)
Fusarium chlamydosporum complex (4)	0 (0)	4 (100)	0 (0)	0 (0)	2 (50)	4 (100)	0 (0)	0 (0)
Fusarium dimerum complex (3)	3 (100)	3 (100)	0 (0)	0 (0)	3 (100)	3 (100)	0 (0)	0 (0)
Fusarium fujikuroi complex (13)	10 (77)	13 (100)	0 (0)	0 (0)	13 (100)	13 (100)	0 (0)	0 (0)
Fusarium incarnatum-equiseti complex (4)	0 (0)	2 (50)	2 (50)	0 (0)	2 (50)	4 (100)	0 (0)	0 (0)
Fusarium oxysporum complex (9)	3 (33)	7 (78)	2 (22.2)	0 (0)	8 (89)	9 (100)	0 (0)	0 (0)
Fusarium solani complex (12)	6 (50)	9 (75)	3 (25)	0 (0)	10 (83)	11 (92)	1 (8.3)	0 (0)
Scedosporium/*Pseudallescheria* spp. (17)	3 (18)	8 (47)	3 (0)	6 (35.3)	16 (94)	17 (100)	0 (0)	0 (0)
Scedosporium apiospermum (5)	2 (40)	3 (60)	2 (40)	0 (0)	4 (80)	5 (100)	0 (0)	0 (0)
Scedosporium aurantiacum (7)	0 (0)	1 (14)	0 (0)	6 (85.7)[d]	7 (100)	7 (100)	0 (0)	0 (0)
Scedosporium boydii (1)	0 (0)	1 (100)	0 (0)	0 (0)	1	1	0 (0)	0 (0)
Scedosporium prolificans (4)	1 (25)	3 (75)	1 (25)	0 (0)	4 (100)	4 (100)	0 (0)	0 (0)
Total (117)	63 (54)	89 (76)	18 (15)	10 (9)	105 (90)	113 (97)	3 (3)	1 (<1)

[a] $n = 117$. ID, identification; mis-ID, misidentification.
[b] Misidentified as *Aspergillus versicolor*.
[c] All *A. fumigatus sensu stricto*.
[d] Misidentified as *Scedosporium apiospermum*.

The Bruker library correctly identified 54% (63/117) of the challenge isolates to the species level and an additional 22% (26/117) to the genus level; all of the 18 isolates not identified were of species not represented in the library (Table 1). When the library was supplemented with the in-house database, identification improved significantly (at a level of $\alpha = 0.01$) with 90% and 96.8% of isolates identified to the species and genus levels, respectively. The median of log scores of the supplemented Bruker library was significantly higher ($\alpha = 0.01$) as was the distribution of scores for individual genera (selected species are represented in Fig. 1). Reductions in the IQRs of scores were evident except for *Aspergillus fumigatus* and *Fusarium oxysporum*. Various proportional increases in species identifications of molds after supplementation of commercial libraries with in-house-built databases have been reported (5–13); Schulthess et al. noted an increase from 52.4% to 79% after supplementation of the same Bruker library (v1.0) (28).

The Bruker library alone identified 69% (38/55) of *Aspergillus* isolates, including all *A. fumigatus sensu stricto* and *Aspergillus flavus*; however, 1/7 *Aspergillus terreus* (represented in the library) and most uncommon species, including *Aspergillus lentulus* (spectra not represented) were not identified (Table 1). The combined database identified 93% of the isolates to the species level except for one (50%) *Aspergillus sydowii* and both *Aspergillus viridinutans* strains. Failure to identify isolates or inaccurate identification (see below) resulted from the absence or limited number of relevant spectra in the Bruker database.

Notably, the largest (5-fold) improvement in identification after database supplementation was for *Scedosporium* (Table 1) with species identification for 94% (16/17) isolates, including all seven *Scedosporium aurantiacum* strains (versus 18% [3/17] by the Bruker library alone). Although *Scedosporium prolificans* is represented in the Bruker library, only 1/4 isolates was identified to the species level. Scedosporiosis is the second most common non-*Aspergillus* mold infection in Australia with 24% of infections due to *S. aurantiacum* (19, 21, 29). Adoption of our database in other Australian centers has the potential to remove reliance on molecular approaches to identify *Scedosporium* to the species level, overcoming the limitations of other dedicated *Scedosporium* databases that utilize different software, thus limiting their wider application (8, 12, 30).

The supplemented Bruker library also identified 2-fold as many *Fusarium* isolates to the species complex level (84% versus 42%; significant at $\alpha = 0.01$) (Table 1). While the Bruker library performed well for the *Fusarium dimerum* complex, it identified only 33 to 50% of the *Fusarium solani* and *Fusarium oxysporum* complexes, the most common causes of fusariosis (31, 32). The

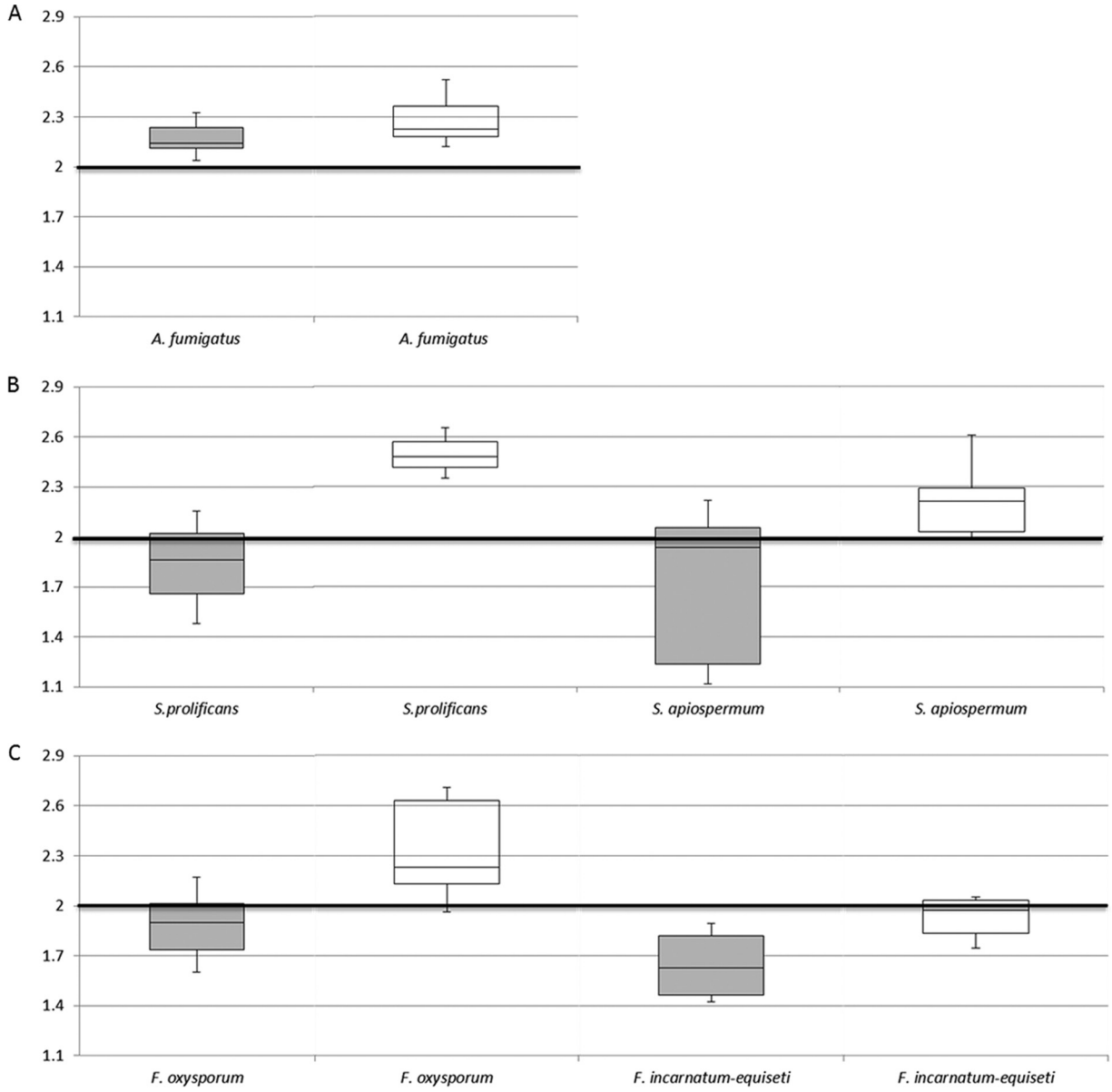

FIG 1 Box and whisker plots illustrating the median mass spectral log scores and interquartile range of scores for *Aspergillus fumigatus* (A), *Scedosporium prolificans* and *Scedosporium apiospermum* (B) and *Fusarium oxysporum* and *Fusarium incarnatum-equiseti* complex (C). Scores achieved when challenged against the Bruker library alone are shown in gray shaded boxes, and those achieved by the combined Bruker library and in-house database are shown in the open boxes. The numbers on the *y* axis represent MALDI-TOF MS log scores.

combined database identified all but one *F. solani* strain, while two isolates each of *Fusarium chlamydosporum* and *Fusarium incarnatum-equiseti* complex had log scores between 1.7 and 1.99 but with correct identification. The challenge of acquiring reproducible spectra for *Fusarium* spp. is noteworthy (5, 9, 26); one study (6) identified only 1/6 *F. solani* complex when interrogated against the same Bruker library (v1.0).

While analyzing *Fusarium* isolates to the species complex level, rather than to the individual species level may be a limitation, higher species-level discrimination may be unnecessary because susceptibility differences between members of the same species complex do not appear to be clinically relevant (26, 33). Species delineation of *Fusarium* necessitates multilocus gene sequence typing (MLST) incorporating at least four loci, and different MLST schemes are recommended for different species complexes (3, 34). Triest et al. (26) built a database of 40 *Fusarium* species where species identification was achieved for 82.8% and 91% of isolates using log scores of ≥2.00 and ≥1.80, respectively, with 97% identified to the species complex level (versus 84% herein).

Lowering the log score cutoff to ≥1.80 for species identification significantly improved identification (69.2% versus 54% of isolates at ≥2.00; $\alpha = 0.01$) by the Bruker library; this difference was significant also for *Fusarium* (42% to 73.3%; $\alpha = 0.01$). While there was also improvement in proportional identification for the combined database, no statistical significance was demonstrated for any of the three genera at the cutoff of ≥1.80 (*Fusarium*; 84%

to 95.6%; *Aspergillus*, 93% to 95%; and *Scedosporium*, 94% to 100%). Improvements in mold identification have been reported, including at cutoffs of ≥1.70 (26, 28) and as low as ≥1.40 using customized databases (12). The modest improvement in species identification observed in our study at a cutoff of ≥1.80 (and even at ≥1.70; data not shown) suggests that the representation of locally relevant spectra in a database, rather than lowering the threshold is more important for test performance.

Twelve isolates (one *Scedosporium apiospermum*, seven *Fusarium*, and four *Aspergillus*) (Table 1) were not identified to the species/species complex level by the combined database but eight had correct identifications at log scores of ≥1.70 to ≤2.00. Of 10 isolates called as misidentifications by the Bruker library, two each were *Aspergillus creber* and *Aspergillus sydowii* (misidentified as *Aspergillus versicolor*) and six were *S. aurantiacum* (as *S. apiospermum*). The combined library called one *A. sydowii* strain as *A. versicolor*. It is possible that identification errors exist in the Bruker database. While we "challenged" the combined database with only 21/28 species with in-house-built spectra, evaluation of other species is ongoing.

In summary, we have developed a clinically relevant database containing 28 species of *Aspergillus*, *Fusarium*, and *Scedosporium*. This library is portable across diagnostic laboratories within Australasia to supplement the Bruker library.

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REFERENCES

1. **Pfaller MA, Diekema DJ.** 2010. Epidemiology of invasive mycoses in North America. Crit Rev Microbiol **36:**1–53. http://dx.doi.org/10.3109/10408410903241444.
2. **Lackner M, de Hoog GS, Verweij PE, Najafzadeh MJ, Curfs-Breuker I, Klaassen CH, Meis JF.** 2012. Species-specific antifungal susceptibility patterns of *Scedosporium* and *Pseudallescheria* species. Antimicrob Agents Chemother **56:**2635–2642. http://dx.doi.org/10.1128/AAC.05910-11.
3. **Wang H, Xiao M, Kong F, Chen S, Dou HT, Sorrell T, Li TY, Xu YC.** 2011. Accurate and practical identification of 20 *Fusarium* species by seven-locus sequence analysis and reverse line blot hybridisation, and an *in vitro* antifungal susceptibility study. J Clin Microbiol **49:**1890–1898. http://dx.doi.org/10.1128/JCM.02415-10.
4. **Bader O.** 2013. MALDI-TOF MS-based species identification and typing in medical mycology. Proteomics **13:**788–799. http://dx.doi.org/10.1002/pmic.201200468.
5. **Iriart X, Lavergne RA, Fillaux J, Valentin A, Magnaval JF, Berry A, Cassaing S.** 2012. Routine identification of medical fungi by the new Vitek MS matrix-assisted laser desorption ionization-time of flight system with a new time-effective strategy. J Clin Microbiol **50:**2107–2110. http://dx.doi.org/10.1128/JCM.06713-11.
6. **Chen Y, Liu Y, Teng S, Liao C, Hung C, Sheng W, Teng L, Hsueh P.** 2015. Evaluation of the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry Bruker Biotype for identification of *Penicillium marneffei*, *Paecilomyces*, *Fusarium solani*, *Rhizopus* species and *Pseudallescheria boydii*. Front Microbiol **6:**679. http://dx.doi.org/10.3389/fmicb.2015.00679.
7. **Alanio A, Beretti JL, Dauphin B, Mellado E, Quesne G, Lacroix C, Amara A, Berche P, Nassif X, Bougnoux ME.** 2011. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for fast and accurate identification of clinically relevant *Aspergillus* species. Clin Microbiol Infect **17:**750–755. http://dx.doi.org/10.1111/j.1469-0691.2010.03323.x.
8. **Sitterlé E, Giraud S, Leto J, Bouchara JP, Rougeron A, Morio F, Dauphin B, Angebault C, Quesne G, Beretti J-L, Hassouni N, Nassif X, Bougnoux M.** 2014. Matrix-assisted laser desorption ionization−time of flight mass spectrometry for fast and accurate identification of *Pseudallescheria*/*Scedosporium* species. Clin Microbiol Infect **20:**929–935. http://dx.doi.org/10.1111/1469-0691.12574.
9. **Marinach-Patrice C, Lethuillier A, Marly A, Brossas J-Y, Gene J, Symoens F, Datry A, Guarro J, Mazier D, Hennequin C.** 2009. Use of mass spectrometry to identify clinical *Fusarium* isolates. Clin Microbiol Infect **15:**634–642. http://dx.doi.org/10.1111/j.1469-0691.2009.02758.x.
10. **De Carolis E, Posteraro B, Lass-Florl C, Vella A, Florio AR, Girmenia C, Colozza C, Tortorano AM, Sanguinetti M, Fadda G.** 2012. Species identification of *Aspergillus*, *Fusarium*, and *Mucorales* with direct surface analysis by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Microbiol Infect **18:**475–484. http://dx.doi.org/10.1111/j.1469-0691.2011.03599.x.
11. **Lau A, Drake S, Calhoun L, Henderson C, Zelazny A.** 2013. Development of a clinically comprehensive database and a simple procedure for identification of moulds from solid media by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol **51:**828–834. http://dx.doi.org/10.1128/JCM.02852-12.
12. **Becker P, de Bel A, Martiny D, Ranque S, Piarroux R, Cassagne C, Detandt M, Hendrickx M.** 2014. Identification of filamentous fungi isolates by MALDI-TOF mass spectrometry: clinical evaluation of an extended reference spectra library. Med Mycol **52:**826–834. http://dx.doi.org/10.1093/mmy/myu064.
13. **Cassagne C, Ranque S, Normand A-C, Fourquet P, Thiebault S, Planard C, Hendrickx M, Piarroux R.** 2011. Mold routine identification in the clinical laboratory by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. PLoS One **6:**e28425. http://dx.doi.org/10.1371/journal.pone.0028425.
14. **Hettick J, Green B, Buskirk A, Kashon M, Slaven J, Janoka R, Blachere F, Schmechel D, Beezhold D.** 2008. Discrimination of *Aspergillus* isolates at the species and strain level by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry fingerprinting. Anal Biochem **380:**276–281. http://dx.doi.org/10.1016/j.ab.2008.05.051.
15. **Gautier M, Ranque S, Normand A-C, Becker P, Packeu A, Cassagne C, Olivier CCL, Hendrickx M, Piarroux R.** 2014. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry: revolutionizing clinical laboratory diagnosis of mould infections. Clin Microbiol Infect **20:**1366–1371. http://dx.doi.org/10.1111/1469-0691.12750.
16. **Normand A-C, Cassagne C, Ranque S, L'Olivier C, Fourquet P, Roesems S, Hendrickx M, Piarroux R.** 2013. Assessment of various parameters to improve MALDI-TOF MS reference spectra libraries constructed for the routine identification of filamentous fungi. BMC Microbiol **13:**76. http://dx.doi.org/10.1186/1471-2180-13-76.
17. **Lackner M, Klaassen CH, Meis J, Gerritsvan Den Ende G, De Hoog GS.** 2012. Molecular identification tools for sibling species of *Scedosporium* and *Pseudallescheria*. Med Mycol **50:**497–508. http://dx.doi.org/10.3109/13693786.2011.618939.
18. **Miceli MH, Lee SA.** 2011. Emerging moulds: epidemiological trends and antifungal resistance. Mycoses **54:**e666–e678. http://dx.doi.org/10.1111/j.1439-0507.2011.02032.x.
19. **Slavin M, Van Hal S, Sorrell TC, Lee A, Marriott DJ, Daveson K, Kennedy K, Hajkowicz K, Halliday C, Athan E, Bak N, Cheong E, Heath C, Morrissey CO, Kidd S, Beresford R, Blyth C, Korman TM, Robinson JO, Meyer W, Chen SC-A.** 2015. Invasive infections due to filamentous fungi other than *Aspergillus*: epidemiology and determinants of mortality. Clin Microbiol Infect **21:**490e1–490e10. http://dx.doi.org/10.1016/j.cmi.2014.12.021.
20. **Morrissey CO, Chen SC, Sorrell TC, Milliken S, Bardy PG, Bradstock KF, Szer J, Halliday CL, Gilroy NM, Moore J, Schwarer AP, Guy S, Bajel A, Tramontana AR, Spelman T, Slavin MA, Australasian Leukaemia Lymphoma Group and the Australia and New Zealand Mycology Interest Group.** 2013. Galactomannan and PCR-directed versus the standard culture and histological-based antifungal strategy for invasive aspergillosis in high-risk haematology patients: a randomised controlled trial. Lancet Infect Dis **13:**519–528. http://dx.doi.org/10.1016/S1473-3099(13)70076-8.
21. **Heath CH, Slavin MA, Sorrell TC, Handke R, Harun A, Philips M, Nguyen Q, Delhaes L, Ellis D, Meyer W, Chen SC, Australian *Scedosporium* Study Group.** 2009. Population-based surveillance for scedosporiosis in Australia: epidemiology, disease manifestations and emergence of

Scedosporium aurantiacum infection. Clin Microbiol Infect **15:**689–693. http://dx.doi.org/10.1111/j.1469-0691.2009.02802.x.

22. **De Hoog GS, Guarro J, Gene J, Figueras MJ.** 2000. Atlas of clinical fungi. The ultimate benchtop tool for diagnostics, version 4.1.2, 4th ed. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
23. **White T, Bruns T, Lee S, Taylor J.** 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, p 315–322. *In* Innis M, Gelfand D, Sninsky J, White T (ed.), PCR protocols: a guide to methods and applications. Academic Press, New York, NY.
24. **Yurkov A, Krueger D, Begerow D, Arnold N, Takka MT.** 2012. Basidiomycetous yeasts from boletales fruiting bodies and their interactions with the mycoparasite *Sepedonium chrysospermum* and the host fungus *Paxillus*. Microb Ecol **63:**295–303. http://dx.doi.org/10.1007/s00248-011-9923-7.
25. **Glass NL, Donaldson G.** 1995. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Appl Environ Microbiol **61:**1323–1330.
26. **Triest D, Stubbe D, De Cremer K, Peirard D, Normand A-C, Piarroux R, Detandt M, Hendrickx M.** 2015. Use of matrix-assisted laser desorption ionization−time of flight mass spectrometry for identification of molds of the *Fusarium* genus. J Clin Microbiol **53:**465–476. http://dx.doi.org/10.1128/JCM.02213-14.
27. **Clinical and Laboratory Standards Institute.** 2008. Interpretative criteria for identification of bacteria and fungi by DNA sequencing; approved guideline. CLSI document MM18-A. Clinical and Laboratory Standards Institute, Wayne, PA.
28. **Schulthess B, Ledermann R, Mouttet F, Zbinden A, Bloemberg G, Bottger E, Hombach M.** 2014. Use of the Bruker MALDI Biotyper for identification of moulds in the clinical mycology laboratory. J Clin Microbiol **52:**2797–2803. http://dx.doi.org/10.1128/JCM.00049-14.
29. **Delhaes L, Arun A, Chen SC, Nguyen Q, Slavin M, Heath CH, Maszewska K, Halliday C, Robert V, Sorrell T, Australian *Scedosporium* (AUSCEDO) Study Group, Meyer W.** 2008. Molecular typing of Australian *Scedosporium* isolates show genetic variability and numerous *S. aurantiacum*. Emerg Infect Dis **154:**282–290.
30. **Coulibaly O, Marinach-Patrice C, Cassagne C, Piarroux R, Mazier D, Ranque S.** 2011. *Pseudallescheria*/*Scedosporium* complex species identification by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Med Mycol **49:**621–626. http://dx.doi.org/10.3109/13693786.2011.555424.
31. **Nucci M, Anaissie E.** 2007. *Fusarium* infections in immunocompromised patients. Clin Microbiol Rev **20:**695–704. http://dx.doi.org/10.1128/CMR.00014-07.
32. **O'Donnell K, Sutton DA, Rinaldi MG, Gueidan C, Crous PW, Geiser DM.** 2009. Novel multilocus sequence typing scheme reveals high generic diversity of human pathogenic members of the *Fusarium incarnatum-F. equiseti* and *F. chlamydosporum* species complexes within the United States. J Clin Microbiol **47:**3851–3861. http://dx.doi.org/10.1128/JCM.01616-09.
33. **Tortorano AM, Prigitano A, Esposto M, Arsenijevic A, Kolarovic J, Ivanovic D, Paripovic L, Klingspor L, Norday I, Hamal P, Candoni A, Caira M, Drogari Apiranthitou M, ECMM Working Group.** 2014. European Confederation of Medical Mycology (ECMM) epidemiological survey on invasive infections due to *Fusarium* species in Europe. Eur J Clin Microbiol Infect Dis **33:**1623–1630. http://dx.doi.org/10.1007/s10096-014-2111-1.
34. **van Diepeningen A, Brankovics B, Iltes J, van der Lee T, Waalwijk C.** 2015. Diagnosis of *Fusarium* infections: approaches to identification by the clinical mycology laboratory. Curr Fungal Infect Rep **9:**135–143. http://dx.doi.org/10.1007/s12281-015-0225-2.