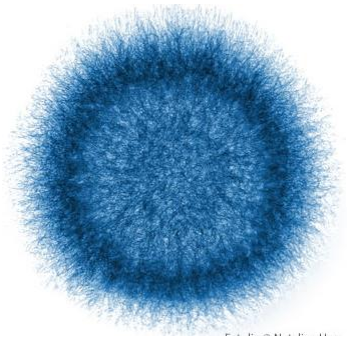
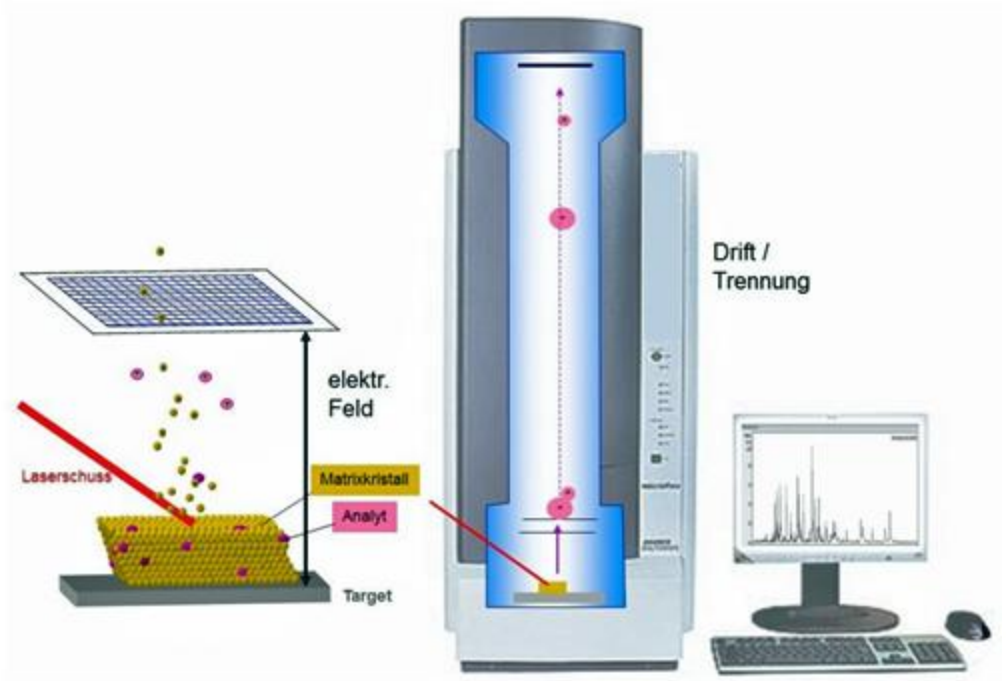
Die Masse machts!?!

Hedda L. Verhasselt

PEG Frühjahrstagung der Sektion Antimykotische Therapie
Bonn, 9.-10. März 2018

Universitätsklinikum Essen

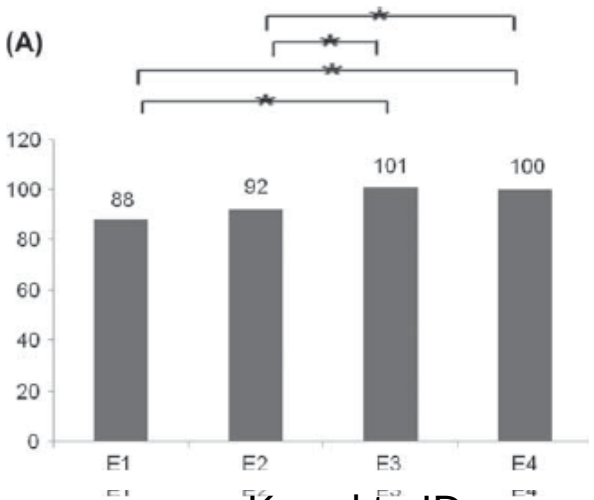




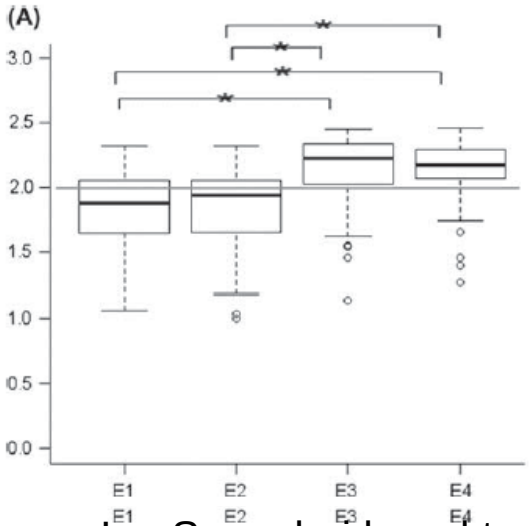
Isolat Extraktion Gerät und Datenbank

http://www.ua-bw.de/pub/beitrag.asp?subid=0&Thema_ID=8&ID=2143

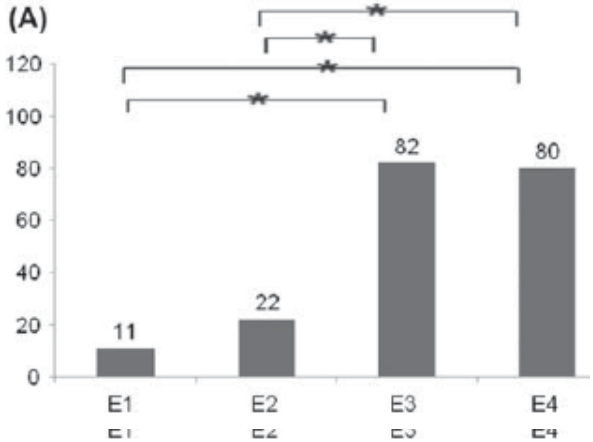
Hefen: Vergleich der Extraktionsmethoden



Korrekte ID



LogScore bei korrekter ID



korrekte ID LogScore ≥ 2

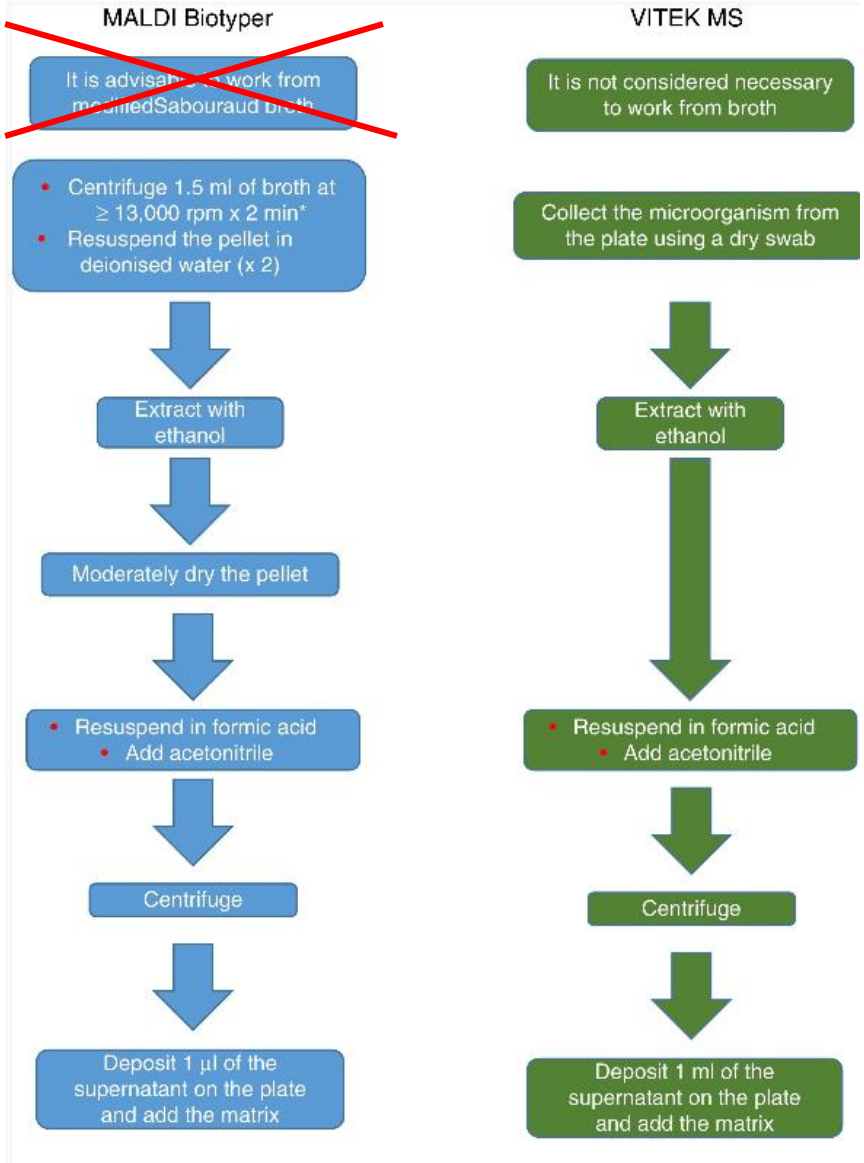
n=103
E1: intact/whole cell MALDI
E2: Ameisensäure
E3: komplette Extraktion (Ethanol, Ameisensäure, Acetonitril)

E4: modifizierte E3

5 min 5 min

Med Mycol. 2013 May;51(4):371-7.

Proteinextraktion Schimmelpilze



VITEK® MS MOULD KIT

REF 415680

R1 = Ethanol* (gebrauchsfertig, 70 %) (4 x 25 ml)

R2 = Ameisensäure** (gebrauchsfertig, 70 %) (4 x 1 ml)

R3 = Acetonitril*** (gebrauchsfertig, 100 %) (4 x 1 ml)

RBT = 2-ml-Röhrchen mit rundem Boden (2 x 50 Einheiten)

Enferm Infecc Microbiol Clin. 2017 May;35(5):303-313

Datenbankeinträge laut Hersteller

Table 1 Composition of the reference databases of the different MALDI-TOF MS platform.

Version	MALDI Biotyper DB5627 + fungi V2	Andromas October 2013	Vitek MS V2.0
No. species of yeast	159 + 0	59	70
No. species of Candida species	82 + 0	40	37
No. species of Cryptococcal species	20 + 0	2	7
No. species of moulds	30 + 130	64	26
No. species of Aspergillus species	7 + 21	24	9
No. species of Fusarium species	2 + 13	2	6
No. species of Scedosporium species	1 + 3	2	0
No. species of Mucorales species	2 + 9	7	0
No. species of dermatophytes species	10 + 15	17	0

Cassagne, Mycoses, 2016, 59, 678–690

Entwicklung der Datenbankeinträge laut Hersteller

Table 1 Composition of the reference databases of the different MALDI-TOF MS platform.

Version	MALDI Biotyper DB5627 + fungi V2	Andromas October 2013	Saramis V4.14.0	Vitek MS V2.0	Vitek MS V3.0
No. species of yeast	159 + 0	59	88	70	83
No. species of Candida species	82 + 0	40	43	37	43
No. species of Cryptococcal species	20 + 0	2	4	7	8
No. species of moulds	30 + 130	64	90	26	79
No. species of Aspergillus species	7 + 21	24	23	9	12
No. species of Fusarium species	2 + 13	2	7	6	7
No. species of Scedosporium species	1 + 3	2	0	0	3
No. species of Mucorales species	2 + 9	7	5	0	6
No. species of dermatophytes species	10 + 15	17	27	0	21

Dimorphe Pilze 0 4

Cassagne, Mycoses, 2016, 59, 678–690

Multicenter Evaluation of the Vitek MS v3.0 System for the Identification of Filamentous Fungi

Jenna Rychert,[a] E. Sue Slechta,[a] Adam P. Barker,[b] Edwin Miranda,[c] N. Esther Babady,[c] Yi-Wei Tang,[c,e] Connie Gibas,[d] Nathan Wiederhold,[d] DeAnna Sutton,[d†] Kimberly E. Hanson[b]

- Multicenter-Studie
- 1,519 Isolate: 26 Genera und 51 Spezies
- Vitek MS v3.0 und Vitek MS mold reagent kit
- Keine in-house Datenbank
- nur in der Datenbank enthaltene Spezies wurden untersucht
- 91% korrekte Spezies-ID, 2% korrekte Genus-ID
- 15 Isolate wurden falsch identifiziert, aber richtiges Genus

TABLE 3 Incorrect identifications for clinical isolates included in the v3.0 database

Identification type	Reference identification[a]	Vitek MS identification[b] (no. of results)
Single identification	*Rhizopus microsporus*	*Rhizopus arrhizus* (1)
	Trichophyton tonsurans	*Trichophyton interdigitale* (2)
	Trichophyton verrucosum	*Trichophyton interdigitale* (3)
	Trichophyton verrucosum	*Trichophyton erinacei* (1)
	Trichophyton violaceum	*Trichophyton rubrum* (5)
	Scedosporium boydii	*Scedosporium apiospermum*[c] (1)
Multiple genera	*Cladophialophora bantiana*	***Cladophialophora bantiana***, *Candida colliculosa* (1)
	Trichophyton violaceum	***Trichophyton violaceum***, *Candida lambica*, *Fusarium oxysporum* complex (1)

[a]For additional analysis regarding the reference identification, see the supplemental material.
[b]Bold indicates correct species identification.

J Clin Microbiol. 2018 Jan 24;56(2).

Insg. 91 Isolate (6%)

TABLE 1 Accuracy of Vitek MS v3.0 compared to sequencing for clinical isolates included in the database

Reference identification	No. (%) with indicated Vitek MS identification			
	Correct to species level	Correct to genus level	Incorrect results	No identification
Dermatophytes (n = 291)				
Arthroderma benhamiae[a] (n = 1)	1 (100)			
Microsporum audouinii (n = 33)	30 (91)	1 (3)		2 (6)
Microsporum canis (n = 31)	30 (97)			1 (3)
Microsporum gypseum (n = 35)	32 (91)			3 (9)
Epidermophyton floccosum (n = 31)	30 (97)			1[b] (3)
Trichophyton mentagrophytes complex[a] (n = 1)	1 (100)			
Trichophyton interdigitale (n = 30)	29 (97)	1 (3)		
Trichophyton rubrum (n = 31)	31 (100)			
Trichophyton tonsurans (n = 33)	30 (91)	1 (3)	2 (6)	
Trichophyton verrucosum (n = 31)	18 (58)	9 (29)	4 (13)	
Trichophyton violaceum (n = 34)	14 (41)	14 (41)	6 (18)	
Total	246 (85)	26 (9)	12 (4)	7 (2)
Dematiaceous (n = 325)				
Alternaria alternata (n = 32)	30 (94)			2 (6)
Curvularia hawaiiensis (n = 26)	25 (96)			1 (4)
Curvularia spicifera (n = 35)	34 (97)			1[b] (3)
Exserohilum rostratum (n = 35)	19 (54)			16[c] (46)
Exophiala dermatitidis (n = 31)	31 (100)			
Exophiala xenobiotica (n = 32)	25 (78)			7 (22)
Scedosporium boydii (n = 32)	30 (94)		1 (3)	1 (3)
Scedosporium apiospermum (n = 41)	41 (100)			
Scedosporium prolificans (n = 32)	32 (100)			
Cladophialophora bantiana (n = 29)	28 (97)		1 (3)	
Total	295 (91)		2 (<1)	28 (9)

J Clin Microbiol. 2018 Jan 24;56(2).

TABLE 1 Accuracy of Vitek MS v3.0 compared to sequencing for clinical isolates included in the database

Reference identification	No. (%) with indicated Vitek MS identification			
	Correct to species level	Correct to genus level	Incorrect results	No identification
Aspergillus species (n = 328)				
Aspergillus brasiliensis (n = 31)	29 (94)			2 (7)
Aspergillus calidoustus (n = 33)	29 (88)			4 (12)
Aspergillus flavus/oryzae (n = 33)	33 (100)			
Aspergillus fumigatus (n = 32)	32 (100)			
Aspergillus lentulus (n = 30)	30 (100)			
Aspergillus nidulans (n = 33)	32 (97)			1 (3)
Aspergillus niger complex (n = 37)	32 (87)			5 (14)
Aspergillus sydowii (n = 30)	30 (97)			1 (3)
Aspergillus terreus complex (n = 34)	32 (94)			2 (6)
Aspergillus unguis[a] (4)	4 (100)			
Aspergillus versicolor (n = 31)	22 (71)	1 (3)		8 (26)
Total	305 (93)	1 (<1)		23 (7)
Other potential pathogens (n = 316)				
Fusarium oxysporum complex (n = 31)	30 (97)			1 (3)
Fusarium proliferatum (n = 30)	30 (100)			
Fusarium solani complex (n = 39)	33 (85)			6 (15)
Paecilomyces variotii (n = 30)	30 (100)			
Penicillium chrysogenum (n = 30)	30 (100)			
Penicillium citrinum[a] (n = 1)	1 (100)			
Rasamsonia argillacea (n = 34)	29 (85)			5 (15)
Acremonium sclerotigenum (n = 30)	30 (100)			
Lecythophora hoffmannii (n = 30)	27 (90)			3 (10)
Sarocladium kiliense (n = 30)	30 (100)			
Purpureocillium lilacinum (n = 31)	29 (94)			2 (6)
Total	299 (95)			17 (5)

J Clin Microbiol. 2018 Jan 24;56(2).

- Zusätzliche 82 Isolate wurden getestet, die nicht in der Datenbank waren
- Keine ID bei n=60
- Inkorrekte ID bei n=22

TABLE 5 Organisms not included in the v3.0 database

Type of identification	Vitek MS identification	Reference identification (no. of results)
Incorrect identification (n = 22)	*Aspergillus flavus/oryzae*	*Aspergillus nomius* (1)
	Aspergillus nidulans	*Aspergillus delacroxii* (3), *Aspergillus quadrilineatus* (3), *Emericella variecolor* (1)
	Aspergillus versicolor	*Aspergillus amoenus* (2), *Aspergillus fructus* (1)
	Candida kefyr/parapsilosis	*Cladophialophora boppii* (1)
	Curvularia hawaiiensis	*Curvularia senegalensis* (1)
	Curvularia spicifera	*Curvularia lunata* (1), *Curvularia pseudolunata* (1)
	Fusarium chlamydosporum complex	*Fusarium incarnatum-equiseti* species complex (1)
	Fusarium oxysporum complex	*Fusarium nygamai* (1)
	Fusarium proliferatum	*Fusarium fujikuroi* (2)
	Mucor velutinosus	*Mucor circinelloides* f. *janssenii* (1)
	Penicillium chrysogenum	*Penicillium rubens* (2)

J Clin Microbiol. 2018 Jan 24;56(2).

Evaluation of the Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System for Identification of Clinically Relevant Filamentous Fungi

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- Single center-Studie
- 319 Isolate, 43 Genera

- Vorgehensweise:
  - Vitek MS v3.0 und SpectraIdentifier R2.1.0 (1)
  - Keine ID→Vergleich mit SARAMIS v4.12 (1+2)

- Extraktion laut Hersteller
- Keine in-house Datenbank
- Auch nicht in der Datenbank enthaltene Spezies wurden untersucht

- (1) 213 (66.8%) korrekte ID
- (1+2) zusätzliche 32 Isolate wurden identifiziert →245 (76.8%) korrekte IDs

J Clin Microbiol. 2016 Aug;54(8):2068-73.

- 103 ohne ID → davon konnte SARAMIS 32 identifizieren
- Von den übrigen 71 ohne ID waren nur 2 Isolate (*A. fumigatus*, *R. microsporus*) in der Datenbank enthalten
- 3 Misidentifizierungen

TABLE 1 Performance of the Vitek MS version 3.0 library for the identification of filamentous fungi

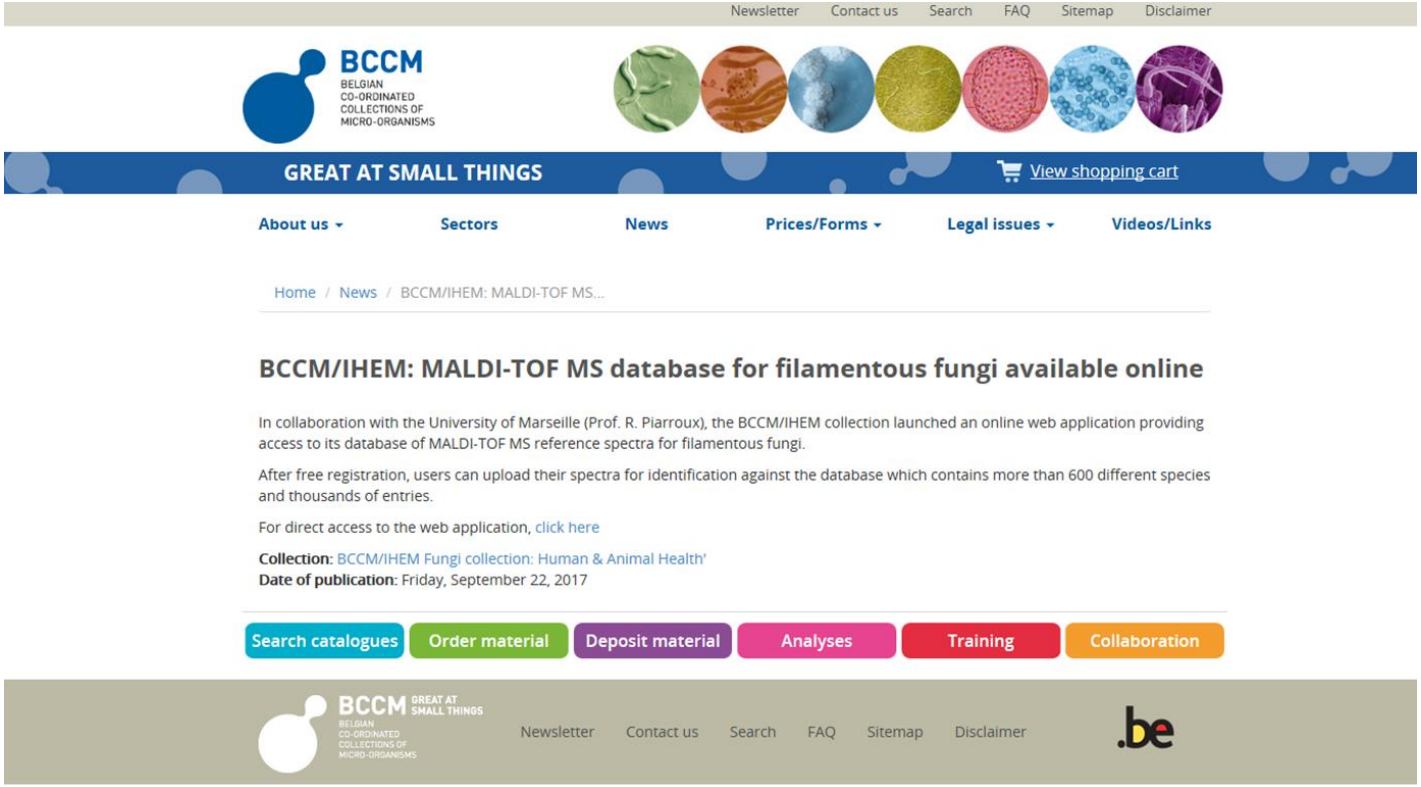
Organism	No. (%) of isolates				
	Total	Identified by Knowledge Base version 3.0	Identified by Knowledge Base version 3.0 plus modified SARAMIS database	Unidentified	Misidentified
Acrodontium salmoneum	1	0 (0.0)	0 (0.0)	1	0
Alternaria spp.	11	10 (90.9)	11 (100)	0	0
Aspergillus spp.	144	125 (86.8)	133 (93.6)	8	3
A. amoenus	2	0 (0.0)	0 (0.0)	0	2
A. calidoustus	1	0 (0.0)	0 (0.0)	0	1
A. carneus	1	0 (0.0)	0 (0.0)	1	0
A. chevalieri	1	0 (0.0)	0 (0.0)	1	0
A. clavatus	2	0 (0.0)	1 (50.0)	1	0
A. flavus	31	28 (90.3)	31 (100.0)	0	0
A. fumigatus	63	61 (96.8)	62 (98.9)	1	0
A. lentulus	2	2 (100.0)	2 (100.0)	0	0
A. nidulans	1	1 (100.0)	1 (100.0)	0	0
A. niger	22	19 (86.3)	22 (100)	0	0
A. sydowii	5	5 (100.0)	5 (100.0)	0	0
A. tennesseensis	1	0 (0.0)	0 (0.0)	1	0
A. terreus	8	8 (100.0)	8 (100.0)	0	0
A. unguis	1	1 (100.0)	1 (100.0)	0	0
A. westerdijkiae	3	0 (0.0)	0 (0.0)	3	0

Profit: Cladosporium, Curvularia, Scopulariopsis

J Clin Microbiol. 2016 Aug;54(8):2068-73.

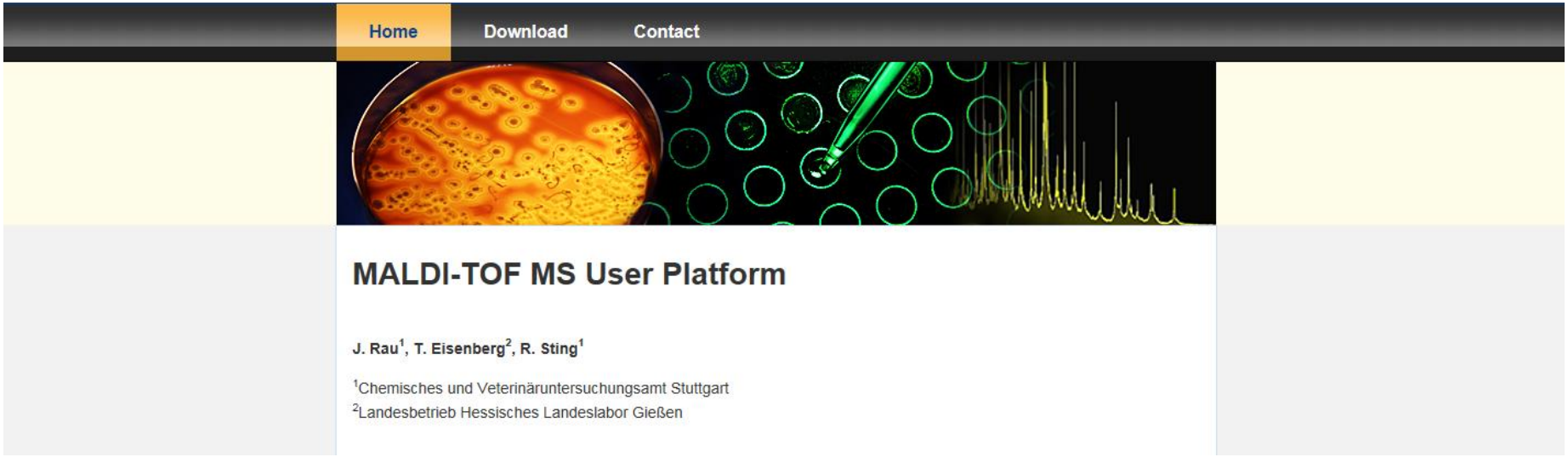
Online-Tool: Pilzdatenbank und Software

- Webseite und Software, die eine Pilzdatenbank mit 938 Spezies und 246 Genera scannen



http://bccm.belspo.be/about-us/bccm-ihem.

J Clin Microbiol. 2017 Sep;55(9):2661-2670.



- Zusammenstellung einer „offenen“ Liste
- Informationen zu Speziesnamen, Asservat sowie Angaben zur Validität der Isolat-Benennung (z.B. molekularbiologische Identifizierung) und zu den technischen Details des Eintrages (Gerät, Kultivierung, Präparation etc.).
- → Überblick über bereits vorhandene Datenbankeinträge
- → Kontaktförderung zum Ersteller/Besitzer des Datenbankeintrages
- **KEINE** Datenbankeinträge zum freien Download hinterlegt

http://maldi-tof-ms-user-platform.ua-bw.de/

Evaluation eines Online-Tools

TABLE 3 Panel 2 identification results obtained with the five identification systems

Result for panel 2 sequenced strains[a]	No. (%) of strains identified				
	IHEM/MRS-MSI (threshold = 20)	IHEM/MRS-MBT (threshold = 1.7)	IHEM/MRS-MBT (threshold = 2.0)	Bruker-MBT (threshold = 1.7)	Bruker-MBT (threshold = 2.0)
Correct at the species level	435 (87.35)	411 (82.53)	312 (62.65)	259 (52.01)	119 (23.9)
Correct at the genus level	26 (5.22)	34 (6.83)	12 (2.41)	41 (8.23)	9 (1.81)
False at the genus level	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Identification criteria not met	37 (7.43)	53 (10.64)	174 (34.94)	198 (39.76)	370 (74.3)

[a]For each database/software combination, the number (%) of strains is specified. Correct, concordant with the molecular identification at either the species or the genus level. False, discordant with the molecular identification at the genus level. Identification criteria not met, score below the defined threshold.

IHEM/MRS=belgisch/französische Pilz-Sammlung
MSI= Online ID Software
MBT= MALDI Biotyper

- "Hoher Schwierigkeitsgrad aufgrund hoher Speziesdiversität und Unterrepräsentation relativ einfach zu identifizierender Stämme (*A.fumigatus*, *A. flavus* und *A. terreus)"*
- **Zeitbedarf:**
  - 5 sec pro Spektrum Bruker-MBT (604 Referenzen)
  - 27 sec pro Spektrum IHEM/MRS-MBT (5,676 Referenzen)
  - 0.14 sec pro Spektrum IHEM/MRS-MSI (etwa 6000 Referenzen)

J Clin Microbiol. 2017 Sep;55(9):2661-2670.

Aspergillus species

Aspergillus species	No. of isolates	No. (%) of isolates with identification results in each indicated range of score values with reference to identification results by molecular methods					No. of references spectra in the Bruker database
		Correct identification to species level				Only correct identification to genus level	
		≥2.000	1.700–1.999	≤1.699	Subtotal		
***ASPERGILLUS* SPECIES COMMONLY ENCOUNTERED ($n \geq 10$)**							
A. fumigatus	107	18 (16.8)	80 (74.8)	8 (7.5)	106 (99.1)	1 (0.9)	12
A. flavus	93	27 (29.0)	51 (54.8)	12 (12.9)	90 (96.8)	3 (3.3)	7
A. niger	75	40 (53.3)	22 (29.3)	11 (14.7)	73 (97.3)	2 (2.7)	12
A. terreus	43	12 (27.9)	16 (37.2)	15 (34.9)	43 (100)	0	10
A. versicolor	11	7 (63.6)	2 (18.2)	1 (9.1)	10 (90.9)	1 (9.1)	10
A. sydowii	11	0	0	0	0	11 (100)	1
A. nidulans	10	6 (60.0)	3 (30.0)	1 (10.0)	10 (100)	0	9
Subtotal	350	110 (31.4)	174 (49.7)	48 (13.7)	332 (94.8)	18 (5.2)	61
***ASPERGILLUS* SPECIES RARELY ENCOUNTERED ($n < 10$)**							
A. tubingensis	7	0	0	0	0	7 (100)	0
A. japonicus[a]	5	0	0	0	0	4 (80.0)	0
A. nomius	4	0	1 (25.0)	2 (50.0)	3 (75.0)	1 (25.0)	1
A. tamarii	3	0	1 (33.3)	0	1 (33.3)	2 (66.7)	1
A. aculeatus	2	0	0	0	0	2 (100)	0
A. ustus	2	1 (50.0)	0	1 (50.0)	2 (100)	0	2
Other species[b]	8	1 (12.5)	0	0	1 (12.5)	7 (87.5)	6[c]
Subtotal	31	2 (6.5)	2 (6.5)	3 (9.6)	7 (22.6)	24 (77.4)	10
Total	381	112 (29.4)	176 (46.2)	51 (13.4)	339 (89.0)	41 (10.8)	71

[a] *One isolate of A. japonicas was identified as Penicillum olsonii with identification score of 1.330.*
[b] *Includes one each of A. oryzae, A. cristatus, A. turcosus, A. caesiellus, A. austroafricanus, A. quadrilineatus, A. unguis, and A. luchuensis.*
[c] *Among these eight species, reference spectra in the Bruker Filamentous library 1.0 are present only for A. unguis (two reference spectra) and A. oryzae (four reference spectra).*

Entscheidungskriterien: Nutzung von Quadruplikaten und Herabsetzung des ID-Scores auf 1.7 verringert die Anzahl unidentifizierter Proben OHNE die Verlässlichkeit zu verändern.

Front Microbiol. 2017 Jun 29;8:1209.

BMC Microbiol. 2017 Jan 31;17(1):25.

Schwierige Aspergillus-Spezies

Table 2
Identification of 345 *Aspergillus* isolates using the MALDI Biotyper, compared with β-tubulin sequence-based identification.

Species complex	β-tubulin sequence-based identification	No. of isolates tested	No (%) of isolates							
			Correct identification[a]		Incomplete identification[b]		No identification		Misidentification	
			Cutoff >2.0	Cutoff >1.7	Cutoff >2.0	Cutoff >1.7	Cutoff >2.0	Cutoff >1.7	Cutoff >2.0	Cutoff >1.7
A. fumigatus complex	*A. fumigatus*	146	140 (95.9)	146 (100)	0	0	6 (4.1)	0	0	0
	A. lentulus[c,d]	2	0	0	0	1 (50)	2 (100)	1 (50)	0	0
A. niger complex	*A. niger*	59	59 (100)	59 (100)	0	0	0	0	0	0
	A. tubingensis[c,d]	21	0	0	21 (100)	21 (100)	0	0	0	0
A. flavus complex	*A. flavus*/*A.oryzae*[e]	52	47 (90.4)	52 (100)	0	0	5 (9.6)	0	0	0
	A. tamarii[c]	2	0	2 (100)	0	0	2 (100)	0	0	0
A. terreus complex	*A. terreus*	44	43 (97.7)	44 (100)	0	0	1 (2.3)	0	0	0
A. vesicolor complex	*A. sydowii*[c]	16	0	0	13 (81.3)	13 (81.3)	0	0	3 (18.8)	3 (18.8)
A. nidulans complex	*A. nidulans*	2	2 (100)	2 (100)	0	0	0	0	0	0
A. ustus complex	*A. calidoustus*[c,d]	1	0	0	1 (100)	1 (100)	0	0	0	0
	Total	345	291 (84.3)	305 (88.4)	35 (10.1)	36 (10.4)	16 (4.6)	1 (0.3)	3 (0.9)	3 (0.9)

[a] Correct identification at the species level.
[b] Incomplete identification included a "low level of discrimination", in which two or three species are proposed, one of which corresponds to sequence-based identification within the same species complex.
[c] Cryptic species identified by β-tubulin sequence analysis.
[d] Not included in the Filamentous Fungi Library 1.0 database (Bruker Daltonik).
[e] Because *Aspergillus flavus* and *Aspergillus oryzae* are difficult to distinguish by β-tubulin sequence analysis, correct identification at the species level was considered if MALDI-TOF indicated *A. flavus* or *A. oryzae*.

Bruker Filamentous Fungi Library version 1.0

Diagn Microbiol Infect Dis. 2017 Jan;87(1):28-31.

Table 1 Identification of 111 clinical isolates by the Bruker library and the Bruker library plus BMU database

Organism (no. of isolates)	No. (%) of isolates at the genus or species level identified by log(score) value							
	Bruker library				Bruker library plus BMU database			
	≥2.0	≥1.7	<1.7	Mis-ID	≥2.0	≥1.7	<1.7	Mis-ID
R. arrhizus (20)	19(95)	20(100)	0(0)	0(0)	19(95)	20(100)	0(0)	0(0)
R. microsporus (27)	24(88.9)	27(100)	0(0)	0(0)	24(88.9)	27(100)	0(0)	0(0)
R. stolonifer (1)	0(0)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)
R. pusillus (4)	4(100)	4(100)	0(0)	0(0)	4(100)	4(100)	0(0)	0(0)
S. racemosum (2)	2(100)	2(100)	0(0)	0(0)	2(100)	2(100)	0(0)	0(0)
L. corymbifera (4)	4(100)	4(100)	0(0)	0(0)	4(100)	4(100)	0(0)	0(0)
L. ramosa (6)	0(0)	3(50)	0(0)	3(50)[a]	0(0)	3(50)	0(0)	3(50)[a]
L. ornata(1)	0(0)	0(0)	0(0)	1(100)[b]	0(0)	0(0)	0(0)	1(100)[b]
M. circinelloides (9)	2(22.2)	6(66.7)	0(0)	3(33.3)[c]	2(22.2)	6(66.7)	0(0)	3(33.3)[c]
M. irregularis (23)	0(0)	0(0)	23(100)	0(0)	23(100)	23(100)	0(0)	0(0)
M. hiemalis (5)	0(0)	0(0)	5(100)	0(0)	3(60)	5(100)	0(0)	0(0)
M. racemosus (4)	0(0)	0(0)	4(100)	0(0)	4(100)	4(100)	0(0)	0(0)
C. bertholletiae (3)	0(0)	0(0)	3(100)	0(0)	3(100)	3(100)	0(0)	0(0)
C. phaeospora (1)	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)
C. echinulata (1)	0(0)	0(0)	1(100)	0(0)	1(100)	1(100)	0(0)	0(0)
Total (111)	55(49.5)	67(60.4)	37(33.3)	7(6.3)	90(81.1)	104(93.7)	0(0)	7(6.3)[d]

Mucorales

- n=111
- Bruker Filamentous Fungi library v1.0 →49.5% korrekte Spezies-ID
- Bruker Filamentous Fungi library v1.0 plus in-house Beijing Medical University (BMU) Datenbank → 81.1%

Mis-ID, misidentification

[a] Misidentified as *Lichtheimia corymbifera*

[b] Misidentified as *Lichtheimia corymbifera*

[c] Misidentified as *Mucor ramosissimus*

[d] Misidentified at the species level but correctly identified at the genus level

J Clin Microbiol. 2018 Feb 7. pii: JCM.01886-17.

Bruker versus Vitek MS

Table 1. Identification results when the microorganism is present in both databases.

Reference identification	Number of isolates	Bruker Biotyper					VITEK MS (IVD)				
		Correct identification to the level of					Correct identification to the level of				
		Species	Genus	Complex/group	No ID	Mis ID	Species	Genus	Complex/group	No ID	Mis ID
Filamentous fungi	17	11	4	0	2	0	7	1	0	8	1
Yeast	28	22	1	0	4	1	26	0	0	2	0
Total number of strains (%)	**380**	**278 (73.2)**	**47 (12.4)**	**19 (5)**	**22 (5.8)**	**14 (3.6)**	**310 (81.6)**	**33 (8.7)**	**9 (2.4)**	**18 (4.7)**	**10 (2.6)**

No ID = No identification obtained. Mis ID = Misidentification obtained

doi:10.1371/journal.pone.0144878.t001

Spezies nicht in der Datenbank

Bruker: 11 Hefen, 28 filamentöse Pilze nicht in Datenbank vorhanden. **Keine** Misidentifikationen.
Vitek MS: 6 Hefen (2 MisID), 51 filamentöse Pilze (**12** Misidentifizierungen, v.a. auf Genusebene)

PLoS One. 2015 Dec 10;10(12):e0144878.

„Weiche“ Faktoren

Table 5. Comparison of practicality and other technical aspects of Microflex LT and Vitek MS.

	Microflex LT	Vitek MS
User friendliness		
Dimensions of device	☺☺☺	☺
Ergonomic design	☺☺	☺
Noise emission	Low	High
Ease of smear preparation	☺☺	☺
Disposable target	No	Yes
Ready-to use matrix solution	No	Yes
Sample capacity per run	16	4x48
Workflow integration*	☺	☺☺
Identification time		
Prepare work list	2 min	2 min
Create vacuum	3 min	6 min
Identification (n° of sample)	50 min (96)	45 min (48)
Results in real-time manner	Per spot	Per 16 spots
Yeasts extraction step	20 min	0
Costs		
Device	☺	☺
Disposables/reactants	☺☺☺	☺☺
Maintenance	☺☺	☺
Other aspects		
Calibration	Every 96 spots	Every 16 spots
Traceability	☺	☺☺
Local technical support*	☺	☺☺
Remote technical support	☺☺	☺☺

☺, acceptable; ☺☺, good; ☺☺☺, very good
*Considering situation in Chile

PLoS One. 2017 May 18;12(5):e0177929.

Candida auris

TABLE 2 Identification by MALDI-TOF MS

Isolate no.	Species tested	Bruker Biotyper: FDA library[a] direct on-plate extraction (score)	Bruker Biotyper: RUO[b] library: Direct on-plate extraction (score)	Bruker Biotyper: RUO[b] library: Full-tube extraction (score)	Vitek-MS: Direct extraction (% identity): IVD[c] library	Vitek-MS: Direct extraction (% identity): RUO library[d]
1	*C. auris*	No ID[e]	*C. auris* (1.75)	*C. auris* (2.19)	No ID	*C. auris* (99)
2	*C. auris*	No ID	No ID	*C. auris* (2.02)	No ID	*C. auris* (98)
3	*C. auris*	No ID	*C. auris* (1.80)	*C. auris* (2.04)	No ID	*C. auris* (99)
4	*C. auris*	No ID	*C. auris* (1.73)	*C. auris* (2.10)	No ID	*C. auris* (99)
5	*C. auris*	No ID	No ID	*C. auris* (1.95)	No ID	*C. auris* (99)
6	*C. auris*	No ID	No ID	*C. auris* (1.97)	No ID	*C. auris* (87)
7	*C. auris*	No ID	No ID	*C. auris* (2.05)	No ID	*C. auris* (99)
8	*C. auris*	No ID	*C. auris* (1.73)	*C. auris* (1.99)	No ID	*C. auris* (99)
9	*C. auris*	No ID	No ID	*C. auris* (2.00)	No ID	*C. auris* (92)
10	*C. auris*	No ID	*C. auris* (1.75)	*C. auris* (1.92)	No ID	*C. auris* (99)
11	*C. duobushaemulonii*	*C. duobushaemulonii* (2.29)	*C. duobushaemulonii* (2.29)	Not tested	No ID	No ID
12	*C. duobushaemulonii*	*C. duobushaemulonii* (2.19)	*C. duobushaemulonii* (2.19)	Not tested	No ID	No ID
13	*C. haemulonii*	*C. haemulonii* (2.29)	*C. haemulonii* (2.29)	Not tested	*C. haemulonii* (99)	*C. haemulonii* (99)
14	*C. duobushaemulonii*	*C. duobushaemulonii* (2.27)	*C. duobushaemulonii* (2.27)	Not tested	No ID	*C. duobushaemulonii* (94)
15	*C. haemulonii*	*C. haemulonii* (2.21)	*C. haemulonii* (2.21)	Not tested	*C. haemulonii* (99)	*C. haemulonii* (99)

[a]The Bruker FDA library contains *C. haemulonii* (12 entries) and *C. duobushaemulonii* (7 entries).
[b]The Bruker RUO library has *C. auris* (three entries).
[c]IVD, *in vitro* diagnostic. The Vitek MS IVD library is an FDA-approved library, and it contains *C. haemulonii*.
[d]The Vitek MS RUO library has *C. auris* and *C. duobushaemulonii*.
[e]ID, identification.

- In beiden IVD-Datenbanken nicht enthalten, aber in beiden RUO
- Bruker: komplette Extraktion ist besser!

J Clin Microbiol. 2017 Feb;55(2):638-640.

ID direkt aus positiven Blutkulturen

- Vitek MS RUO mit SARAMIS v.4.12 plus in-house Datenbank
- in-house Protein Extraktion:
  - 4-ml Blut+ 0.5% Natriumlaurylsulfat
  - Zentrifugation, Sediment waschen bis blutfrei
  - Komplette Extraktion
- Quadruplikate, valide ID bei Konfidenzlevel ≥75%

Table 2. SARAMIS™ identification results of mass spectra from positive blood cultures and from colonies, and final molecular identification.

	Identification from blood culture aliquot		Identification from colonies		
Case No.	SARAMIS 1st match (%)	SARAMIS 2nd match (%)	SARAMIS 1st match (%)	SARAMIS 2nd match (%)	Pairwise sequence alignment (%, GenBank accession no.)
1	*Exophiala* sp. (95.7)	*Exophiala dermatitidis* (76.6)	*Exophiala* sp. (96)	*Exophiala dermatitidis* (80)	*Exophiala dermatitidis* (100, AY663828.1)
2	*Saprochaete clavata*[1] (80)	–	*Saprochaete clavata*[1] (99.7)	*Saprochaete clavata*[1] (99.8)	*Saprochaete clavata* (100, KF984489.1)
3	*Fusarium* sp. (92.3)	*Fusarium verticillioides* (75.5)	*Fusarium* sp. (99.9)	*Fusarium verticillioides* (79.4)	*Fusarium verticillioides* (99, KR905553.1)
4	*Trichosporon asahii* (99.9)	*Trichosporon asahii* (95)	*Trichosporon asahii* (99.9)	*Trichosporon asahii* (96.5)	*Trichosporon asahii* (100, KR265116.1)
5	*Fusarium solani*[2] (99.9)	*Fusarium solani*[2] (92.4)	*Fusarium solani*[2] (99.9)	*Fusarium solani*[2] (89.5)	*Fusarium solani* (100, LN827973.1)
6	*Fusarium solani*[2] (82.5)	*Fusarium solani*[2] (75.9)	*Fusarium solani*[2] (83.2)	*Fusarium solani*[2] (76.8)	*Fusarium solani* (99, HM852038.1)
7	*Trichosporon asahii* (99.9)	*Trichosporon asahii* (95)	*Trichosporon asahii* (99.9)	*Trichosporon asahii* (95.0)	*Trichosporon asahii* (100, KR265116.1)

[1]Assigned as *Geotrichum clavatum* by the SARAMIS™ database
[2]Identification by in-house SuperSpectrum.

Med Mycol. 2016 Nov 1;54(8):885-9.

MALDI-TOF MS as a rapid screening method for azole-resistance in *Aspergillus fumigatus* sensu stricto

P0004

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Objective

The emergence of azole resistance in *Aspergillus fumigatus* is of clinical concern. However, azole susceptibility testing by broth microdilution takes at least 48 hours, which is a critical time lag in the treatment of patients with invasive aspergillosis.

Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry (MS) arranges the proteome of a microorganism in a peak pattern of peptides with increasing mass-charge ratio, disclosing a characteristic spectrum.

Resistant *A. fumigatus* strains with a tandem repeat in the CYP51A gene are described to overexpress the encoded protein 14-α-demethylase[1], which could hypothetically lead to a change in the generated peak pattern compared to azole-susceptible *A. fumigatus*. If resistant and susceptible strains could be discriminated by MALDI-TOF MS, this approach would speed up resistance detection and impact patient management.

Methods

- Azole-resistant and intermediate *A. fumigatus* strains from our culture collection are submitted to MALDI-TOF MS.
- The resulting spectra are compared with spectra from an equal number of susceptible strains.
- Resistance in *A. fumigatus* (R) is defined as a strain with resistance to at least one tested azole. Intermediate (I) susceptibility is defined when an intermediate MIC to at least one tested azole is observed. (MIC interpretative criteria (mg/L): Itraconazole: S <2, I =2, R >2; Voriconazole: S <2, I =2, R >2; Posaconazole S <0,5, I =0,5, R >0,5).
- All experiments are performed on the Biotyper instrument from Bruker Daltonics Inc. (Germany).
- Optimal spectra are obtained from *Aspergillus* cultured from diluted Sabouraud agar slants, after formic acid extraction.

Results and Discussion

The preliminary results presented here include the data obtained from 42 azole-resistant *A. fumigatus* isolates and 23 *A. fumigatus* strains with an intermediate susceptibility. The spectra of these strains were compared to 68 clinical azole-susceptible *A. fumigatus* strains (table 1).

Included spectra		CYP51A genotype
Resistant	N=42	28 TR34/L98H 12 TR46/Y121F/T289A 1 wild type (CYP51A-unrelated mechanism) 1 TR53
Intermediate	N=23	15 wild type (CYP51A-unrelated mechanism) 7 F46Y/M172V/E427K 1 P375T
Susceptible	N=68	

Table 1. Included *A. fumigatus* strains for MALDI TOF MS analysis

Two separate clusters of spectra are identified

→ A "susceptible" cluster containing 49/68 of the susceptible isolates and 20/23 intermediate isolates versus only 3/42 resistant isolates.

→ The second "resistant" cluster contains 39/42 spectra originating from resistant isolates.

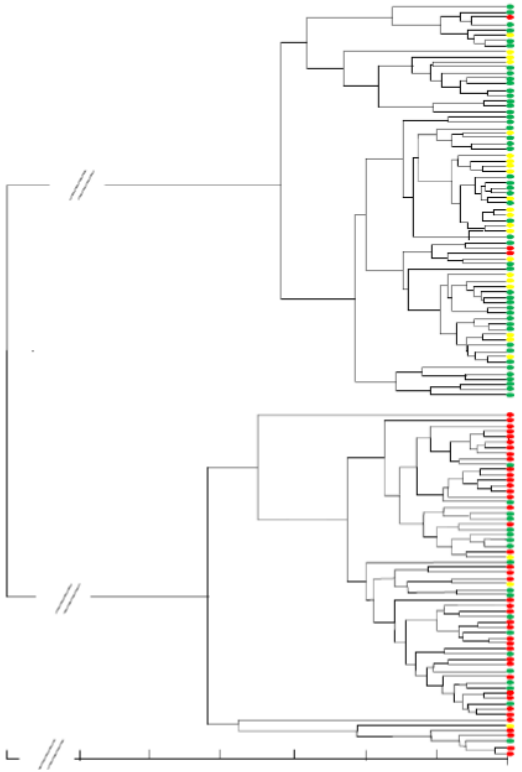
→ MALDI TOF MS is able to discriminate resistant and susceptible strains ($p<0.05$). **MALDI TOF MS identifies the resistant strains with a sensitivity of 93% and a specificity of 77%.**

In our center, the prevalence of azole resistance is 5.5% among clinical isolates. As a result of the lower specificity, the application of MALDI TOF MS as a screening tool for resistance would therefore lead to a positive predictive value of only 18% for resistance when a spectrum clusters in the resistant branch. However, when a spectrum is placed in the "susceptible branch", resistance can be excluded with a **negative predictive value of 99%**

Conclusions

MALDI TOF MS appears to be a promising tool to exclude azole resistance in clinical *A. fumigatus* isolates. The prospective analysis of clinical isolates will demonstrate if this approach is reproducible and if these promising results can be confirmed.

Figure 1. MALDI TOF MS generated dendrogram. Red dots: azole-resistant strains. Yellow dots: azole intermediate strains. Green dots: azole-susceptible.



References

[1] Mellado et al. New *Aspergillus fumigatus* Resistance Mechanism Conferring In Vitro Cross-Resistance to Azole Antifungals Involves a Combination of *cyp51A* Alterations. Antimicrob Agents Chemother 2007; 51(6):1897-1904

ECCMID 2014

Proof-of-concept Studie

- **Frage 1:** Kann MALDI-TOF MS die minimale Konzentration an Voriconazol, die eine Profiländerung im Spektrum verursacht (MPCC~ MHK), bestimmen?
- **Frage 2:** Kann diese MPCC *Aspergillus spp.*-Isolate als WT oder non-WT klassifizieren?
- **Frage 3:** Hat diese Methode Vorteile gegenüber traditioneller BMD?

TABLE 1 Results of antifungal susceptibility testing of *Aspergillus* spp. to voriconazole using genotyping, BMD MIC, and MALDI-TOF MS MPCC determination

Strain	Species	Cyp51A amino acid substitution	BMD MIC (μg/ml) at 48 h (WT identification)	MALDI-TOF MS MPCC (μg/ml) at: 24 h			30 h			48 h		
				Trial[a] 1	Trial 2	WT identification	Trial 1	Trial 2	WT identification	Trial 1	Trial 2	WT identification
F13747	*A. fumigatus*	G434C/G138C	4 (non-WT)	4	0.5	ND[b]	2	2	Non-WT	2	2	Non-WT
F15122	*A. fumigatus*	G448S	4 (non-WT)	8	16	Non-WT	8	16	Non-WT	16	8	Non-WT
F17294	*A. fumigatus*	L98H+TR[c]	4 (non-WT)	4	2	Non-WT	2	2	Non-WT	8	8	Non-WT
F11628	*A. fumigatus*	G138C	4 (non-WT)	0.5	2	ND	2	2	Non-WT	2	2	Non-WT
Af958	*A. fumigatus*	WT	0.12 (WT)	0.5	0.25	WT	0.5	0.25	WT	1	0.5	WT
Af982	*A. fumigatus*	WT	0.12 (WT)	0.25	0.125	WT	0.25	0.25	WT	0.25	1	WT
Af983	*A. fumigatus*	WT	0.12 (WT)	0.5	0.25	WT	0.25	0.125	WT	0.5	0.25	WT
Af987	*A. fumigatus*	WT	0.25 (WT)	0.5	1	WT	1	0.25	WT	1	0.25	WT
Af919	*A. fumigatus*	WT	0.12 (WT)	0.25	0.25	WT	0.5	0.125	WT	0.5	0.25	WT
Af921	*A. fumigatus*	WT	0.12 (WT)	0.5	0.25	WT	0.25	0.125	WT	0.5	0.25	WT
Af928	*A. fumigatus*	ND[b]	0.25 (WT)	0.125	0.125	WT	0.25	0.25	WT	0.125	0.25	WT
Af484	*A. fumigatus*	ND	0.125 (WT)	0.125	0.25	WT	0.25	0.25	WT	0.125	0.25	WT
Af804	*A. fumigatus*	ND	0.25 (WT)	0.25	0.25	WT	0.5	0.5	WT	0.5	0.25	WT
Au204	*A. ustus*	ND	8	4	4	WT	8	4	WT	8	16	WT
Af748	*A. fumigatus*	ND	0.25 (WT)	0.25	0.25	WT	0.5	0.25	WT	0.5	0.25	WT
Au960	*A. ustus*	ND	8	8	8	WT	8	8	WT	16	8	WT
Af829	*A. fumigatus*	ND	0.25 (WT)	0.25	0.125	WT	0.25	0.25	WT	0.25	0.25	WT
Ac366	*A. calidoustus*	ND	8	8	4	WT	8	8	WT	16	16	WT
Af608	*A. fumigatus*	ND	0.25 (WT)	0.25	0.125	WT	0.125	0.125	WT	0.25	0.25	WT
Af163	*A. fumigatus*	ND	0.5 (WT)	0.25	0.25	WT	0.5	0.25	WT	0.5	0.5	WT

[a]"Trial" indicates a biological replicate.
[b]ND, not determined.
[c]Tandem repeat mutations in combination with substitutions at codon L98.

J Clin Microbiol. 2017 Jul;55(7):2030-2034.

Zusammenfassung

- Erhöhen die Erfolgsquote: komplette Extraktion, Quadruplikate und Herabsetzung des ID-Scores auf 1.7 (Bruker)
- Kommerzielle Datenbanken benötigen Optimierung
- Zugang zu RUO-Datenbanken sollte erleichtert werden, Online Tools sind verfügbar
- Antimykotika-Empfindlichkeitstestung mittels MALDI-TOF MS funktioniert.

Table 4. Comparison of antimicrobial resistance prediction methods

Features		WGS-based prediction	Microarray-based prediction	MS-based prediction
Biological aspects	Target	DNA	cDNA	Protein
	Target stability	Medium	Medium	High
	Background	Dead cells, salt, human DNA	rRNA	Human protein
	Direct sample analysis	Yes (urine)	No	Yes (urine)
	Phenotypic inference	Partial	Partial	Quasi-total
	Ability to detect target mutations	Appropriate	Not easily	To be demonstrated
	Postanalysis[a)]	Yes	No	Yes
Technological aspects	Method	WGS	Microarray	LC-ESI-MS/MS (DIA approach[d)])
	Sample preparation automation	POP[e)]	Yes	POP
	Analysis automation	Yes	Yes	Yes
	Multiplexing capacity	Yes	Not easily	Yes
	Quantification capacity	No	Low	High
	Skills needed to operate	Medium	Low	High
	System miniaturization	Yes (POP)	Yes	Yes (POP)
	System maintenance	Medium	Low	High
Performance	Agreement with AST[b)]	Yes	POP	POP
	Real MIC[c)]	No	No	No
	Time to result	8–24 h	6 h	2 h
	Throughput	Low	Medium	Medium
	Development costs	Medium	High	Medium
	Instrument costs	Medium	Low	High
	Reagent costs	High	High	Medium
	Upgrade flexibility	High	Low	High
	Requires more than 10^5 cells	No	Yes	Yes
	Single-cell analysis	POP	POP	POP

a) Ability to process data a posteriori.
b) Antibiotic susceptibility testing.
c) Minimum inhibitory concentration.
d) Data-independent acquisition.
e) Proof-of-principle.

Instruments for Clinical MALDI

- Linear MALDI-TOF preferred for most clinical applications
  - Simple, reliable, robust, and very sensitive over wide mass range
  - Reproducible spectra with wide dynamic range
  - Adequate mass resolving for higher mass proteins and oligos
  - Resolving power and mass accuracy may be insufficient for some applications to peptides and small molecules
- Reflector MALDI-TOF provides higher resolving power and mass accuracy
  - Allows identification by mass fingerprinting at low mass (e.g tryptic digests)
  - Provides accurate mass for input to MS-MS identification
- MALDI MS-MS

Vitek MS: Achtung!

Organismen mit niedrigerer Identifizierungsgenauigkeit

WICHTIG: Bestätigen Sie die Identifizierung dieser Organismen mit einer weiteren Methode:

- ***Trichophyton rubrum***

Identifizierung nicht benannter Spezies

Es besteht die Möglichkeit einer Kreuz-Identifizierung zwischen den folgenden von **VITEK® MS** angezeigten Spezies und nicht in der Datenbank enthaltenen Spezies.

Angezeigte Spezies	Mögliche nicht in der Datenbank enthaltene Spezies
Aspergillus flavus / oryzae	*Aspergillus nomius*
Aspergillus nidulans	*Emericella variecolor*
Aspergillus calidoustus	*Aspergillus ustus*
Fusarium proliferatum	*Fusarium fujikuroi*
Mycobacterium intracellulare	*Mycobacterium chimaera*
Mycobacterium mucogenicum	*Mycobacterium phocaicum*
Nocardia beijingensis	*Nocardia asiatica*
Penicillium chrysogenum	*Penicillium rubens*

Angezeigte Identifizierung für Subspezies, Spezies oder Speziesgruppen		Subspezies oder Spezies	Alter Name in Wissensdatenbank V2.0
Aspergillus flavus/oryzae	(3)	*Aspergillus flavus*	
		Aspergillus oryzae	
Cryptococcus albidus	(4)	*Cryptococcus albidus*	
		Cryptococcus albidus var albidus	
Fusarium oxysporum complex	(2)	*Fusarium oxysporum*	*Fusarium oxysporum*
		Fusarium oxysporum f.sp aechmeae	
		Fusarium oxysporum f.sp cyclaminis	

(2) Speziesgruppe ist die endgültige Identifizierung. Die Subspezies oder Spezies in dieser Gruppe sind zur Information aufgeführt.

(3) Subspezies oder Speziesgruppe wird als Keimpaar-Befund angezeigt. Alle Subspezies- oder Speziesnamen sind zur Information aufgeführt.

(4) Die Subspezies oder Speziesgruppe ist eine einzeln identifizierte Subspezies oder Spezies, obwohl andere Subspezies oder Spezies in die Identifizierung mit eingeschlossen werden. Die anderen in die Identifizierung eingeschlossenen Subspezies oder Spezies sind zur Information aufgeführt.

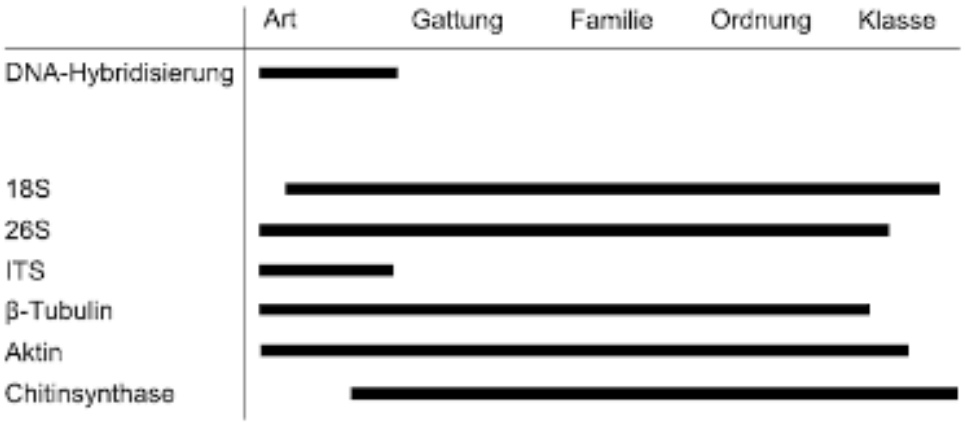


Abb. 8.4 Phylogenetische und taxonomische Einordnung von Pilzisolaten aufgrund von genotypischen Methoden (DNA-Hybridisierung, Sequenzierung von rRNA-Partialsequenzen, ITS-Regionen und verschiedenen Protein-Genen von diagnostischer Bedeutung). Für die Identifizierung von Pilzarten und -stämmen haben sich die DNA-Hybridisierung und die variablen ITS-Regionen bewährt. Für die taxonomische Einordnung von Pilzisolaten sind zudem morphologisch-physiologische und genetische Merkmale erforderlich