Impact of MALDI-TOF on the diagnosis of invasive fungal infections

Maurizio Sanguinetti
Institute of Microbiology
Università Cattolica del Sacro Cuore – Rome – Italy
Faculty disclosure

• Invited speaker:
  Pfizer, Gilead, MSD, Astellas, Bruker Daltonics, Becton Dickinson

• Consultant: Pfizer, Gilead, MSD, Thermo Fisher

• Research Grants:
  Pfizer, Gilead, MSD, BioMerieux, Becton Dickinson, BioRad
The MALDI-TOF process

- Spots to be analyzed are shot by a laser, desorbing and ionizing microbial and matrix molecules from the target plate.
- The cloud of ionized molecules is accelerated into the TOF mass analyzer, toward a detector.
- Lighter molecules travel faster, followed by progressively heavier analytes.
- A mass spectrum is generated, representing the number of ions hitting the detector over time. Separation is by mass-to-charge ratio, but because the charge is typically single for this application, separation is effectively by molecular weight.

MALDI-TOF MS applications

• Clinical isolates identification

• Direct identification of pathogens in clinical samples

• Subtyping

• Drug susceptibility testing
MALDI-TOF MS is a powerful tool for clinical laboratory identification of human pathogenic yeasts.

<table>
<thead>
<tr>
<th>Study</th>
<th>No. isolates/species</th>
<th>% isolates identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stevenson et al., 2010</td>
<td>194/23</td>
<td>87.1 (99)a</td>
</tr>
<tr>
<td>Bader et al., 2011</td>
<td>1192/36</td>
<td>97.6</td>
</tr>
<tr>
<td>Dhiman et al., 2011</td>
<td>138/14</td>
<td>92.0 (96.3)a</td>
</tr>
<tr>
<td>Goyer et al., 2012</td>
<td>335/17</td>
<td>94.0</td>
</tr>
<tr>
<td>Bille et al., 2012</td>
<td>162/20</td>
<td>98.8</td>
</tr>
<tr>
<td>Iriart et al., 2012</td>
<td>192/18</td>
<td>81.4</td>
</tr>
<tr>
<td>Sendid et al., 2013</td>
<td>1207/28</td>
<td>97.5</td>
</tr>
<tr>
<td>Lacroix et al., 2013</td>
<td>1383/20</td>
<td>98.3</td>
</tr>
<tr>
<td>Westblade et al., 2013</td>
<td>852/31</td>
<td>96.1</td>
</tr>
<tr>
<td>Mancini et al., 2013</td>
<td>157/30</td>
<td>92.3 (87.3)b</td>
</tr>
<tr>
<td>Van Herendael et al., 2013</td>
<td>167/22</td>
<td>97.6c</td>
</tr>
</tbody>
</table>

Reproducible and accurate, with low consumable costs ($0.50 per sample) and minimal preparation time (5 min of hands-on time per identification)

Several closely related species (e.g., Candida ‘psilosis’ or Candida glabrata/bracarensis) could be resolved by MALDI-TOF MS, but not by a biochemical approach

---

a Using a score threshold of ≥1.8. b The percentage in parenthesis was obtained with the Vitek MS system; using the Bruker Biotyper and an in-house-extended database, the success rate increased from 92.3 (as indicated) to 100%. c According to a protocol based on a simplified extraction procedure and lower identification threshold
Multicenter Study Evaluating the Vitek MS System for Identification of Medically Important Yeasts

Laia F. Westblade,*,† Rebecca Jernemann,‡ John A. Brandt,*, Maureen Bythrow,*, Mary Jane Ferraro,*, Omar G. Garell,‡ Christine C. Girocho,‡ Michael A. Lewinske,‡ Ryhana Matsui,∗ A. Brian Mochen,† Gary W. Procop,‡ Sandia S. Richter,‡ Jenna A. Rychert,‡ Linda Serca,∥ Carey-Arm D. Bumitum**

July 2013 Volume 51 Number 7

**TABLE 1** Performance characteristics of the Vitek MS system in identifying clinically relevant *Candida* species

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total</th>
<th>Identified correctly to genus</th>
<th>Identified correctly to species</th>
<th>Unidentified</th>
<th>Misidentified</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>58</td>
<td>57 (98.3)</td>
<td>57 (98.3)</td>
<td>0 (0)</td>
<td>1 (1.7)*</td>
</tr>
<tr>
<td><em>Candida dubliniensis</em></td>
<td>34</td>
<td>34 (100)</td>
<td>34 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida famata</em></td>
<td>29</td>
<td>29 (100)</td>
<td>29 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>62</td>
<td>62 (100)</td>
<td>62 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida guilliermondii</em></td>
<td>36</td>
<td>35 (97.2)</td>
<td>35 (97.2)</td>
<td>1 (2.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida haemulonii</em></td>
<td>12</td>
<td>12 (100)</td>
<td>12 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida inconspicua</em></td>
<td>23</td>
<td>23 (100)</td>
<td>23 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida intermedia</em></td>
<td>7</td>
<td>7 (100)</td>
<td>7 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida kefyr</em></td>
<td>30</td>
<td>30 (100)</td>
<td>30 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida krusei</em></td>
<td>53</td>
<td>53 (100)</td>
<td>53 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida lambica</em></td>
<td>0</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>9 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida lipolytica</em></td>
<td>28</td>
<td>28 (100)</td>
<td>28 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida lusitaniae</em></td>
<td>33</td>
<td>30 (90.9)</td>
<td>29 (87.9)</td>
<td>3 (9.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida metapsilosis</em></td>
<td>30</td>
<td>29 (96.7)</td>
<td>29 (96.7)</td>
<td>1 (3.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>73</td>
<td>72 (98.6)</td>
<td>72 (98.6)</td>
<td>0 (0)</td>
<td>1 (1.4)*</td>
</tr>
<tr>
<td><em>Candida pelliculosa</em></td>
<td>33</td>
<td>33 (100)</td>
<td>33 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida rugosa</em></td>
<td>6</td>
<td>6 (100)</td>
<td>6 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>54</td>
<td>51 (94.4)</td>
<td>49 (90.7)</td>
<td>3 (5.6)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida utilis</em></td>
<td>8</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>Candida zeylanoides</em></td>
<td>8</td>
<td>8 (100)</td>
<td>8 (100)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Total 626 616 (98.4) 612 (97.8) 8 (1.3) 2 (0.3)

*a* Isolate misidentified as *C. dubliniensis.*  
*b* Isolate misidentified as *C. pelliculosa.*
We report the first comparative evaluation between the Bruker Biotyper MS (BMS) and the Vitek MS (VMS) for the identification of yeasts. Correct identification rate at the species level was comparable using the commercial databases (89.8% vs. 84.3% p=0.712), but higher for BMS using an in-house-extended database (100% vs. 84.3% p=0.245).

Importantly, the rate of misidentification was significantly higher for VMS (1% vs. 12.1% p<0.0001), including the rate of major errors (0% vs. 4.5% p=0.0036).
Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry-Based Method for Discrimination between Molecular Types of Cryptococcus neoformans and Cryptococcus gattii

Brunella Postorano, Antoniotta Valia, Massimo Cogliati, Elena De Caroile, Ada Rita Florio, Patrizia Fortuna, Maurizio Sanguinetti, and Anna Maria Tortorino

Institute of hygiene, University of Camerino, Camerino, Italy; Institute of Microbiology, University of Camerino, Camerino, Italy; Institute of hygiene, University of Camerino, Camerino, Italy; Institute of hygiene, University of Camerino, Camerino, Italy; and Clinical Laboratory, Spedali Civili, Bologna, Italy

TABLE 1. Comparison between identification results obtained by MALDI-TOF MS analysis and DNA-based methods for 82 challenge and 25 reference isolates of C. neoformans and C. gattii

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Molecular characterization</th>
<th>MALDI-TOF MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species</td>
<td>Molecular type</td>
</tr>
<tr>
<td>IUM 97-4877</td>
<td>C. neoformans</td>
<td>aA</td>
</tr>
<tr>
<td>IUM 98-3592</td>
<td>C. neoformans</td>
<td>aA</td>
</tr>
<tr>
<td>IUM 97-4315</td>
<td>C. neoformans</td>
<td>aB</td>
</tr>
<tr>
<td>IUM 98-0977</td>
<td>C. neoformans</td>
<td>aA</td>
</tr>
<tr>
<td>IUM 98-2450</td>
<td>C. neoformans</td>
<td>aA</td>
</tr>
<tr>
<td>IUM 98-3410</td>
<td>C. neoformans</td>
<td>aA</td>
</tr>
<tr>
<td>IUM 98-4848</td>
<td>C. neoformans</td>
<td>aA</td>
</tr>
<tr>
<td>IMU 201313</td>
<td>C. neoformans</td>
<td>aA</td>
</tr>
<tr>
<td>CR 38</td>
<td>C. neoformans</td>
<td>aA</td>
</tr>
<tr>
<td>CR 49</td>
<td>C. neoformans</td>
<td>aB</td>
</tr>
<tr>
<td>CR 52</td>
<td>C. neoformans</td>
<td>aB</td>
</tr>
<tr>
<td>IUM 91-3233</td>
<td>C. neoformans</td>
<td>aD</td>
</tr>
<tr>
<td>IUM 94-2361</td>
<td>C. neoformans</td>
<td>aD</td>
</tr>
<tr>
<td>IUM 93-3922</td>
<td>C. neoformans</td>
<td>aD</td>
</tr>
<tr>
<td>CR 33</td>
<td>C. neoformans</td>
<td>aD</td>
</tr>
<tr>
<td>CR 35</td>
<td>C. neoformans</td>
<td>aD</td>
</tr>
<tr>
<td>IUM 93-4041</td>
<td>C. neoformans</td>
<td>aD</td>
</tr>
<tr>
<td>IUM 91-1871</td>
<td>C. neoformans</td>
<td>aAD</td>
</tr>
<tr>
<td>IUM 92-6892</td>
<td>C. gattii</td>
<td>aB</td>
</tr>
<tr>
<td>IUM 91-6402</td>
<td>C. gattii</td>
<td>aB</td>
</tr>
<tr>
<td>WM 163</td>
<td>C. gattii</td>
<td>aB</td>
</tr>
<tr>
<td>IUM 92-6997</td>
<td>C. gattii</td>
<td>aB</td>
</tr>
<tr>
<td>IUM 94-6315</td>
<td>C. gattii</td>
<td>aB</td>
</tr>
<tr>
<td>IP 189</td>
<td>C. gattii</td>
<td>aC</td>
</tr>
<tr>
<td>WM 137</td>
<td>C. gattii</td>
<td>aC</td>
</tr>
<tr>
<td>NMIM 155</td>
<td>C. gattii</td>
<td>aC</td>
</tr>
<tr>
<td>WM 779</td>
<td>C. gattii</td>
<td>aC</td>
</tr>
<tr>
<td>NMIM 103</td>
<td>C. gattii</td>
<td>aC</td>
</tr>
</tbody>
</table>

The only one isolate with discordant results is marked in bold.
Cluster analysis of MALDI-TOF MS spectra of selected reference or challenge isolates of *C. neoformans* and *C. gattii*
Except for 23 isolates, MALDI results were validated without additional phenotypic or molecular tests. Thus, we feel that our strategy can enhance the rapidity and accuracy of MALDI-TOF MS in identifying medically important yeast species.
In-house protocol extraction: detergent plus yeast protocol extraction
Starting material: 6 ml of the blood culture fluid
From specimen extraction to final result: 25 min
The study was conducted between November 2012 and October 2013 in the clinical microbiology laboratory of the Catholic University Medical Center. Overall 1198 BSIs (1053 monomicrobial and 145 polymicrobial) were evaluated.
The Bruker BioTyper provided isolate IDs that were fully concordant with standard culture-based ones for 93.6% (986/1053) of the isolates, with mean (IQR) \( \log(score) \) values for the best hits of 2.09 (1.99-2.20). The Bruker MALDI BioTyper broth analysis yielded no ID for 61 organisms, while 6 isolates were misidentified.

The FilmArray furnished correct IDs for 85% (57 of 67) of those.

Using the combined approach 99% (1043 of 1053) of monomicrobial BSIs were identified.

From: Spanu and Sanguinetti, unpublished
Overall, the Bruker BioTyper furnished **full concordant IDs** with the reference method for 3 (2.1%) BSIs, partial concordance for **92 (63.4%)** and no results for **50 (34.5%)**.

From: Spanu and Sanguinetti, unpublished
Our preliminary data suggest that the new workflow that integrates MALDI-TOF MS and FilmArray allows rapid and accurate detection of bacteria and yeasts responsible for BSIs.

**FilmArray increased the rate of correct BSI IDs provided by MALDITOF-MS from 82.5% (989 of 1198) to 98% (1179 of 1198)**

<table>
<thead>
<tr>
<th>Gram-negative only (N=29)</th>
<th>Gram-positive only (N=23)</th>
<th>Gram-negative/Gram-positive (N= 65)</th>
<th>Bacteria/yeasts (N=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.5%</td>
<td>8.7%</td>
<td>6.1%</td>
<td>3.6%</td>
</tr>
</tbody>
</table>

- Overall, the FilmArray furnished full concordant IDs with the reference method for 136 (93.8%) BSIs, partial concordance for 8 (5.5%) and no results for one episode (0.7%).
- Identification of KPC, mecA, vanA, and vanB as markers of antibiotic resistance.
- The FilmArray assay correctly detected all isolates harboring \(bla_{KPC}\) (n = 15), \(mec\) (n=59) and \(van\) (n=1) genes, with no false positive results.

From: Spanu and Sanguinetti, unpublished
Old and new workflow for rapid identification of BSIs at the clinical microbiology laboratory of Gemelli hospital

BSI, bloodstream infection; ID, identification; AST, antimicrobial susceptibility testing; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry
Impact of Rapid Organism Identification via Matrix-Assisted Laser Desorption Ionization Time-of-Flight Combined with Antimicrobial Stewardship Team Intervention on Adult Patients with Bacteremia and Candidemia

Angela M. Huang, Duane Newton, Anjly Keswani, Tejal N. Gandhi, Lorraine L. Winker, Jacqueline Leip, Curtis D. Collins, Jered L. Nagel
### Table 3: Clinical and Treatment-Related Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Pre-Intervention (n=256)</th>
<th>Intervention (n=245)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical outcomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-day all-cause mortality (%)</td>
<td>52 (20.3)</td>
<td>31 (12.7)</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>Time to microbiological clearance (days)*</td>
<td>3.5 ± 4.8</td>
<td>3.3 ± 5.7</td>
<td>0.928</td>
<td></td>
</tr>
<tr>
<td>Length of hospitalization (days)†</td>
<td>14.2 ± 20.6</td>
<td>11.4 ± 12.9</td>
<td>0.066</td>
<td></td>
</tr>
<tr>
<td>Length of ICU stay (days)‡</td>
<td>14.9 ± 24.2</td>
<td>8.3 ± 9.0</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Recurrence of same BSI (%)</td>
<td>15 (5.9)</td>
<td>5 (2.0)</td>
<td>0.038</td>
<td></td>
</tr>
<tr>
<td>30-day readmission with same BSI (%)</td>
<td>9 (3.5)</td>
<td>4 (1.6)</td>
<td>0.262</td>
<td></td>
</tr>
<tr>
<td><strong>Treatment-related outcomes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to effective therapy (hours)*</td>
<td>30.1 ± 67.7</td>
<td>20.4 ± 20.7</td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>Time to optimal therapy (hours)*</td>
<td>90.3 ± 75.4</td>
<td>47.3 ± 121.5</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± Standard Deviation
† Length of hospitalization and ICU stay were defined as time from blood culture positivity to discharge
‡ Length of hospitalization was defined as time from blood culture positivity to discharge
Total hospitalization costs per patient decreased, on average, by $19,547 during the intervention period compared with the preintervention group. In our 1000-bed quaternary care hospital, we project a cost savings of ~$18 million annually with the implementation of this strategy for the management of gram-negative BSIs.
TABLE 3 Estimated annual costs for the standard protocol and MALDI protocol

<table>
<thead>
<tr>
<th>Item</th>
<th>Standard protocol cost ($)</th>
<th>MALDI protocol cost ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual reagent costs</td>
<td>158,645.33</td>
<td>29,613.84</td>
</tr>
<tr>
<td>Annual labor costs (based on reagent time)</td>
<td>31,323.98</td>
<td>26,669.34</td>
</tr>
<tr>
<td>Fixed annual costs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>of MALDI protocol</td>
<td></td>
<td>31,272.53</td>
</tr>
<tr>
<td>Total</td>
<td>189,969.31</td>
<td>87,555.70</td>
</tr>
</tbody>
</table>

* See Table S5 in the supplemental material.

** The total costs for all items are shown in boldface type.
Is MALDI-TOF MS-based identification really adoptable for routine fungal diagnostics?

- Protein patterns expected to change in response to growth conditions
- Differences and similarities in mass spectral patterns are or not are consistent with the established taxonomy
- Lack of comprehensive databases covering all clinically relevant species

From: Welker M. *Proteomics*. 2011; 11:3143–3153
Is MALDI-TOF MS-based identification really adoptable for routine fungal diagnostics?

It was speculated that highest concordance between acquired (experimental) spectra and the reference spectra in each system’s library could be reached

PROVIDED THAT

the sample preparation procedure used for the MALDI-TOF MS system at hands does not differ from that used to construct the system’s reference library (i.e., Biotyper, Saramis, Andromas)

Confirming earlier observations, no isolates were misidentified. Those 33 isolates failing identification (score of <1.7), either were basidiomycetes (25 isolates) not associated with clinical disease or were *Penicillium* (8 isolates) species not represented in the database.

Once again, the Bruker’s original cutoff scores of ≥2.0 for species and ≥1.7 for genus-level identifications could be maintained, so that specificity was not compromised to improve sensitivity.
<table>
<thead>
<tr>
<th>References</th>
<th>Origin of isolates</th>
<th>MALDI system (database\textsuperscript{a})</th>
<th>Species studied (+ not in DB)</th>
<th>Acceptance criteria for ID</th>
<th>Isolates with positive IDs (+ not in DB)</th>
<th>Accuracy (%)\textsuperscript{b}</th>
<th>Comments</th>
<th>Reference method(s)\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bille [31]</td>
<td>France</td>
<td>Andromas (v. 2010)</td>
<td>6</td>
<td>≥65 %</td>
<td>63/64</td>
<td>98.4</td>
<td></td>
<td>MB\textsuperscript{c}</td>
</tr>
<tr>
<td>Iriart [32]</td>
<td>France</td>
<td>Vitek MS (v. 1)</td>
<td>3 (+6)</td>
<td>–</td>
<td>36/44 (+8)</td>
<td>81.8</td>
<td>8/44 isolates excluded</td>
<td>MB</td>
</tr>
<tr>
<td>Cassagne [28]</td>
<td>France</td>
<td>Biotyper (in-house)</td>
<td>6 (+4)</td>
<td>≥1.7</td>
<td>119/119 (+5)</td>
<td>100</td>
<td>5/124 isolates excluded</td>
<td>MO/MB\textsuperscript{d}</td>
</tr>
<tr>
<td>Lau [33]</td>
<td>USA</td>
<td>Biotyper (in-house)</td>
<td>18\textsuperscript{e}</td>
<td>≥2.0</td>
<td>123/127</td>
<td>98.4</td>
<td>2/127 isolates scored between 1.7 and 1.99</td>
<td>MO/MB\textsuperscript{d}</td>
</tr>
<tr>
<td>Alanio [34]</td>
<td>France</td>
<td>Andromas (in-house)</td>
<td>24</td>
<td>≥66 %</td>
<td>138/140</td>
<td>98.6</td>
<td>–</td>
<td>MB</td>
</tr>
<tr>
<td>De Carolis [35]</td>
<td>Italy</td>
<td>Biotyper (in-house)</td>
<td>14</td>
<td>≥2.0</td>
<td>78/81</td>
<td>96.3</td>
<td>3/81 isolates scored between 1.7 and 1.99</td>
<td>MB</td>
</tr>
</tbody>
</table>

All studies, except for the Alanio et al.’s study [34] that also included 16 hospital environmental isolates, were conducted prospectively by testing clinical isolates and are listed based on the order by which they are mentioned in the text.

\textit{ID} identification, \textit{DB} database, \textit{MB} molecular biology, \textit{MO} morphology

\textsuperscript{a} “In-house” denotes those studies where own self-made libraries were initially constructed and validated, and then challenged alone or in combination with the respective system’s commercial database. Otherwise, the commercial library version was specified.

\textsuperscript{b} To assess the rate of correct identifications by MALDI-TOF MS, reference identification methods, such as sequencing of \(\beta\)-tubulin and/or calmodulin gene regions (MB) [9] and morphological analyses (MO) [8], were used.

\textsuperscript{c} For one isolate that yielded no “good identification” after two runs of MALDI-TOF MS, proper identification was achieved using MB analysis.

\textsuperscript{d} Discrepancies between MALDI-TOF MS results and MO identifications were resolved by MB analyses; if the last results confirmed those of MALDI-TOF MS, the isolates were considered correctly identified by MALDI-TOF MS, regardless of the MO identification results.

\textsuperscript{e} Of 127 isolates (from a total of 421 molds tested), one was identified as \textit{Aspergillus} species by MO analysis alone.
An engineered database included species-specific spectral fingerprints of young and mature colonies of reference strains. The performance of this database was evaluated on 124 clinical isolates and 16 environmental *Aspergillus* isolates, resulting in a 98.6% correct identification. No isolate was misidentified (specificity: 100%) MALDI-TOF MS proved to be a powerful tool for fast and accurate identification of *Aspergillus* species isolated in clinical practice.
<table>
<thead>
<tr>
<th>Order</th>
<th>Genus</th>
<th>Strain*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eurotiales</td>
<td>Aspergillus</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Scopularis</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucorales</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lichtheimii</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhizopus</td>
<td></td>
</tr>
<tr>
<td>Hypocreales</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pantherosporus</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- Reference spectra at different stages of maturation:
  - $10^9$ from young colonies
  - $10^9$ from mature colonies (diameter >3 cm)
- 67 reference strains of **Eurotiales**
- 42 reference strains of **Mucorales and Hypocreales**

Mold Identification by MALDI: Challenge of 103 clinical isolates

- MALDI-TOF MS identified, according to their designated species, 91 of 94 clinical isolates (96.8%) of *Aspergillus*, *Fusarium*, and *Mucorales*.

- The log(score) values of the 91 isolates with correct results were all higher than 2.0, whereas three isolates with a log(score) value of <2.0 (1.817, 1.874, and 1.796, respectively) could be identified only to the genus level; although had concordant species designations as compared with the results of the reference method.

- By contrast, isolates belonging to the species not included in our database (9 isolates: 3 *Alternaria alternata*, 2 *Scedosporium prolificans*, 2 *Curvularia*, 1 *Beauveria bassiana*, 1 *Cladosporium*) had all log(score) values of <1.7, thereby confirming the specificity of MALDI-TOF MS identification.

Cluster analysis of MALDI-TOF spectra of selected reference strains and challenge isolates (CH15, CH25 and CH51) identified as *Aspergillus* section *Flavi* species

As several *A. flavus* isolates are known to be non(afla)toxigenic (and more similar to *A. oryzae* than to other *A. flavus* isolates), our findings raise the possibility of using this approach for discriminating toxigenic from atoxigenic *A. flavus* strains

Trend del n. di isolati e di specie di funghi filamentosi negli ultimi 10 anni presso il Policlinico A. Gemelli

Altezza delle colonne indica il numero di isolati
Colori indicano il numero di specie

Courtesy A. Vella
Despite high species “complexity” in the *Aspergillus* section *Flavi*, we further demonstrated that MALDI-TOF is sufficiently robust to support species level identification of clinical mold isolates in a timely and straightforward manner.
Fusarium solani species complex subtyping

The strains were clustered in separate groups according to their phylogenetic species designation.

- Each MSP is compared with the other in a matrix of cross-wise identification values. The matrix is used to calculate the distance values for each pair.
- Based on the protein mass patterns, strains can be clustered hierarchically.
SUSCEPTIBILITY DETERMINATION

Microdilution methods

MIC determination

E-Test

Disk diffusion

It could take 48 h
End-point subjective

SUSCEPTIBILITY DETERMINATION

Mass spectrometry

MEC

New generation of susceptibility tests

?
MPCC, the minimal profile change concentration

New endpoint:
a value defined as the lowest drug concentration at which a mass spectrum profile change can be detected

MPCC determinations were concordant with MIC BDM irrespective of the type of drug resistance mechanism

- Inoculum $10^6$ yeast/ml
- Turnaround time 15 h
- Acid extraction
- End-point reading objective
- Cost less than 1 euro
We developed a MALDI-TOF MS-based assay for testing antifungal susceptibilities of *Candida* and *Aspergillus* species to the echinocandin caspofungin, by relying on the proteome changes which are detectable after a 15-h exposure of fungal cells to serial drug concentrations.
By means of a composite correlation index (CCI)-based approach, the method reliably and accurately allows to determine the minimal profile change concentration (MPCC), an endpoint value that is an alternative to the classical MIC (Marinach et al. 2009).

To date, while the endpoint readings achievable with MALDI-TOF MS represent a slight time-saving (15 h versus 24 h) over the CLSI/EUCAST method with respect to Candida species, MALDI-TOF MS has the great advantage of eliminating subjective read-outs which occur with the CLSI (and EUCAST) method when filamentous fungi, such as Aspergillus species, are tested.
Rapid Antifungal Susceptibility Testing by Matrix-Assisted Laser Desorption Ionization—Time of Flight Mass Spectrometry Analysis

Comparison by the obtained CCI and susceptibility assessment

Creation of a Composite Correlation Index (CCI)

Target preparation

Extraction by formic acid and ethanol

Spectra production

By MALDI-TOF MS

3h at 37°C in agitation

[0 µg/ml] [0.03 µg/ml] [32 µg/ml]
Performances of the ms-AFST method for 62 clinical *C. albicans* isolates according to the presence (R) or absence (S) of *FKS1* hot-spot mutation

<table>
<thead>
<tr>
<th>Method</th>
<th>Species</th>
<th>No. of isolates</th>
<th>No. of misclassified isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total (f/z WT)</td>
<td>CA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ms-AFST</td>
<td><em>C. albicans</em></td>
<td>62 (11/51)</td>
<td>61/62 (98.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VME&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1/62 (1.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ME&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0/62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mE&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NA&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CLSI</td>
<td><em>C. albicans</em></td>
<td>62 (11/51)</td>
<td>61/62 (98.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>VME&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0/62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ME&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0/62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>mE&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1/62 (1.6)</td>
</tr>
</tbody>
</table>

<sup>a</sup>CA: categorical agreement; VME: very major error; ME: major error; mE: minor error; NA: not applicable
MALDI-TOF intact cell MS (Pros)

- Rapidity
- Inexpensiveness in terms of labor and consumables
- High discriminatory power, accuracy, and superiority over morphological analysis and comparable to molecular identification
- Ability to easily differentiate species that are morphologically and phylogenetically similar to each other
MALDI-TOF intact cell MS (Cons)

- MALDI-TOF MS equipment is not cheap
- Molecular diagnostic techniques are still required in cases for which no reference spectra are present in the MALDI-TOF MS databases at the time of analysis
- Apart from positive blood cultures and urine, MALDI-TOF cannot yet be used directly on patient samples
- Also, the system is not able to identify the presence of several different pathogens in a sample
CONCLUSIONS (1)

• MALDI-TOF identification still requires a growth step in order to obtain fungal colonies for the acquisition of spectra.

• Apart from positive blood cultures, MALDI-TOF cannot yet used directly on patient samples.

• Also, the system is not able to identify the presence of several different pathogens in a sample.
CONCLUSIONS (2)

- In addition to the identification process, other aspects of microorganism analysis, such as the search for virulence factors and drug resistance determinants, and typing, will be expanded enormously.

- This, in combination with the potential of each laboratory to create its own reference database to be widely used and shared, will help to extend MALDI-TOF analysis in clinical mycology laboratories.
SPECIAL THANKS TO:

Istituto di Microbiologia, Università Cattolica del Sacro Cuore, Roma, Italy
E. De Carolis
B. Posteraro
A. Vella
A. R. Florio
R. Torelli
L. Vaccaro
T. Spanu
G. Fadda

Innsbruck Medical University, Innsbruck, Austria
C. Lass-Flörl

Public Health Research Institute, New Jersey, USA
D. S. Perlin

Dipartimento di Biotecnologie Cellulari ed Ematologia,
Università La Sapienza di Roma, Italy
C. Girmenia
C. Colozza

Dipartimento Sanità pubblica Microbiologia e Virologia,
Università degli Studi di Milano, Italy
M. Cogliati
A. M. Tortorano