MALDI-TOF MS
Educational quizz

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Conflict of interests?

- In Lausanne, we use a Bruker mass spectrometer but we have no conflict of interest with Bruker

Relationship with industry
- Ongoing research grant with SUEZ-ONDEO (France)
- Research grant until December 2016 with BD-Kiestra (USA)
1. What is Maldi-tof?
2. Bacterial identification
3. Typing
4. Carbapenemase activity detection
5. Quality issues
6. Costs issues
7. Conclusions
1. What is MALDI-TOF?

Q1. Does “M” means “medical” in the abbreviation MALDI? (“medical applications of laser desorption/ionisation”)

YES (correct)
I DO NOT KNOW
NO (false)
1. What is MALDI-TOF?

Q1. Does “M” mean “medical” in the abbreviation MALDI? (“medical applications of laser desorption/ionisation”)

NO
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

Croxatto, Prod’hom & Greub, FEMS Microbiol Reviews 2011
1. What is MALDI-TOF?

Q2. Regarding the matrix, is it generally basic?

YES (correct)
I DO NOT KNOW
NO (false)

www.studylib.net
1. What is MALDI-TOF?

Q2. Regarding the matrix, is it generally basic?

**NO**, for its application in bacteriology we use an acidic matrix to detect ribosomal proteins that are abundant & basic.

**E. coli**

<table>
<thead>
<tr>
<th>Ribosomal proteins</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL36</td>
<td>4364.33</td>
</tr>
<tr>
<td>RS32</td>
<td>5095.62</td>
</tr>
<tr>
<td>RL34</td>
<td>5380.39</td>
</tr>
<tr>
<td>RL33meth.</td>
<td>6255.39</td>
</tr>
<tr>
<td>RL32</td>
<td>6315.19</td>
</tr>
<tr>
<td>RL30</td>
<td>6410.60</td>
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<tr>
<td>RL35</td>
<td>7157.74</td>
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<tr>
<td>RL29</td>
<td>7273.46</td>
</tr>
<tr>
<td>RL31</td>
<td>7871.66</td>
</tr>
<tr>
<td>RS21</td>
<td>8368.76</td>
</tr>
</tbody>
</table>

Suh et al 2004
1. What is MALDI-TOF?

**Commonly used matrices**

- 2,5 dihydroxybenzoic acid (DHB)
- Alpha-cyano-4-hydroxycinnamic acid (CHCA)
- Sinapinic acid (SA)
- Ferulic acid (FA)
- 2,4 hydroxyphenylbezoic acid

Glycoproteins
- glycopeptides

Proteins

1.5 microl CHCA in 50% acetonitrile/2.5% trifluoroacetate
1. What is MALDI-TOF?

Q3. Regarding the Bruker score, is it a log scale?

YES (correct)
I DO NOT KNOW
NO (false)
1. What is MALDI-TOF?

Q3. Regarding the Bruker score, is it a log scale?

**YES**, 2 means that there are 10% of expected matches (100 out of 1000),
Thus, reducing the criteria from 1.7 to 1.5 is huge

Score = function (database; spectra; ...)

Score > 2
Score 1.7-2
Score < 1.7
Q4. Does the commercial Bruker database includes all the major human pathogens?

YES (correct)
I DO NOT KNOW
NO (false)
1. What is MALDI-TOF?

Q4. Does the commercial Bruker database include all the major human pathogens?

Anthrax infection in an injecting drug user in Germany
Anthrax was not suspected initially; the patient died

- Day 1: Patient admitted to the hospital
  - Blood cultures sent to the laboratory
  - Patient dies due to septic shock
  - Blood cultures positive with Gram-positive bacilli (late afternoon)

- Day 2: Growth of *Bacillus* spp. on subcultures
  - MALDI-TOF: *Bacillus cereus*
  - Discussions on anthrax suspicion
  - Different PCRs and 16S sequencing over night

- Day 3: *B. anthracis* confirmed by PCR

Identified as *B. cereus* on day 1
Retrospectively identified as *B. anthracis*

Identified by PCR on day 3

Importance of Using Bruker's Security database

20 BSL3 pathogens:
- *Burkholderia pseudomallei*
- *Brucella suis*, *Brucella melitensis*
- *Francisella tularensis*

Without Security database, no reliable identification for 18/20 isolates despite high quality spectra

Cunningham SA & Patel R.

Useful to use a database that includes BSL3 pathogens to avoid further work-up and exposure risk
Q5. I stands for ionisation .... Does all ionisation approaches help detecting peptides and/or proteins?

YES (correct)
I DO NOT KNOW
NO (false)
1. What is MALDI-TOF?

Ionization methods

1. Plasma desorption (PD)
2. Fast atom bombardment (FAB)
3. Chemical ionization (CI)
4. Atmospheric pressure CI
5. Laser desorption
6. Electrospray ionization (ESI)
7. MALDI

Croxatto, Prod’hom & Greub, FEMS Microbiol Reviews 2011
1. What is Maldi-tof?
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Q6. Although the ribosomal proteins are generally used for bacterial identification using the MALDI-TOF approach, is it true that several additional proteins may be detected such as glutaredoxin?

YES (correct)
I DO NOT KNOW
NO (false)
1. What is MALDI-TOF?

**About 50% of detected proteins are ribosomal proteins**

Others proteins (*Salmonella*) include:

- Cold shock-like protein (Csph)
- Translation initiation factor IF-1
- Ribosome modulation factor
- Integration host factors A & B
- Nucleoid-associated proteins H-NS
- RNA chaperone CspeE
- Glutaredoxin-1
- Phosphocarrier protein HPr

**= abundant and basic**

2. Identification

Q7. With MALDI-TOF is it possible to identify bacteria such as *Actinomyces, Streptomyces, Actinobaculum* and *Nocardia* that are poorly identified with routine phenotypic methods?

YES (correct)
I DO NOT KNOW
NO (false)
3. Identification of “Difficult to identify strains”

Q7. With MALDI-TOF is it possible to identify bacteria such as Actinomyces, Streptomyces, Actinobaculum and Nocardia that are poorly identified with routine phenotypic methods?

YES, but need a specific extraction protocol

Isolates identified at the species level by 16S rDNA sequencing (n=419)

- Concordant ID at species level (n=241) 57.5%
- score x ≥ 2.0 (n=182) 43.4%
- score 1.7 ≤ x ≤ 2.0 (n=59) 14.1%
- No ID or incorrect ID (n=178) 42.5%

Streptomyces, Actinomyces and Nocardia: need a specific extraction protocol

Improved identification of Gram positive rods

- Improved database
- Improved extraction protocol
- Changed cut-off (1.7 instead 2)

Reduction of unidentified Gram positive rods from 12.1% to 5-6%

3. Identification of “Difficult to identify strains”

**Specific extraction protocols for mycobacteria, nocardia, actinomycetes & yeasts**

- **Mycobacteria**
  1. Transfer bacteria to screw cap tube containing water and detergent (Tween)
  2. Heat at 95° for 1 hour to inactivate bacteria
  3. Add Formic Acid/ Acetonitrile mixture

- **Nocardia, Actinomycetes**
  1. Boil large amounts of bacteria (turbid suspension)
  2. Ethanol extraction, dry pellets, and resuspend in Formic Acid

- **Yeast**
  1. Culture 24 to 72 hours depending upon fungal species and media type
  2. Select colony
  3. Spot target organism, overlay with 1μL Formic Acid
  3. Full extraction with Ethanol and Formic Acid/Acetonitrile

Clark, Kaleta, Arora, Wolk. Clinical Microbiology Reviews 2013
Q8. When using MALDI-TOF on blood culture pellet with only an in situ protein extraction, do we generally easily identify *Klebsiella pneumoniae*?

- YES (correct)
- I DO NOT KNOW
- NO (false)
Q8. When using MALDI-TOF on blood culture pellet with only an in situ protein extraction, do we generally easily identify *Klebsiella pneumoniae*? NO

**Factors possibly affecting MALDI-TOF MS efficiency**

Presence of a capsule
- *K. pneumoniae, H. influenzae, S. pneumoniae* (n=13)

Close relatedness of bacteria within streptococci
- *S. pneumoniae, S. oralis, ...* (n=13)

Cell wall composition of Gram positive bacteria conferring high resistance to lysis (n=21)

Presence of residual blood proteins ?

Inadequate procedure ?

2. Identification

Q9. Is it possible to identify strict intracellular bacteria directly from cell culture?

- YES (correct)
- I DO NOT KNOW
- NO (false)
2. Identification

Q9. Is it possible to identify strict intracellular bacteria directly from cell culture? YES

In amoebae (chlamydiae)

- Rickettsia
  Yssouf et al. PLOS Negl Trop Dis 2015

- Borrelia
  Fotso et al. PLOS Negl Trop Dis 2015

In ticks

- Rickettsia
- Borrelia

Lienard et al. Microbes and Infection 2011
2. Identification

Q10. Is it possible to identify mosquito using MALDI-TOF?

YES (correct)
I DO NOT KNOW
NO (false)
2 Identification

Q10. Is it possible to identify mosquito using MALDI-TOF?

YES

Ticks (*I. ricinus, R. sanguineus, D. marginatus*)
even when ticks are infected with rickettsia, anaplasma or borrelia
Yssouf et al. Journal Clin Microbiol 2013

Mosquito (*Anopheles, Aedes, Culex, ..*)
147/149 correctly identified
Yssouf et al. PLOS One 2013; Parasitol research 2014

Fleas
Yssouf et al. Comp Immunol Microbial Infect Dis 2014

Culicoides (diptera)
437 culicoides from 10 species
Sambou et al. JCM 2015
Q11. When applied to blood cultures pellet, the MALDI-TOF has a clinical impact mainly on the antibiotic treatment (i.e. streamlining and broadening) ?

YES (correct)
I DO NOT KNOW
NO (false)
2. Clinical impact of MALDI-TOF

Q11. When applied to blood cultures pellet, the MALDI-TOF has a clinical impact only on antibiotic treatment change (i.e. start of ABtt, streamlining, broadening, ....) ?

**NO**

**Impact of MALDI-TOF**

- 21/197 results led to a treatment change
- New blood cultures (n=2)
- Catheter removal (n=4)
- Additionnal investigations (n=3)
- Documented sample exchange (n=1)
- Exclude a contamination

Martiny et al. Clin Microbiol Infect 2013
2. Bacterial identification

Q12. Shorter time to reporting of the antibiotic susceptibility results from the use of MALDI-TOF on blood culture pellet?

YES (correct)
I DO NOT KNOW
NO (false)
Q12. Shorter time to reporting of the antibiotic susceptibility results from the use of MALDI-TOF on blood culture pellet?

**NO**
2. Identification

Q13. Is it possible to identify virulence factors using MALDI-TOF…

YES (correct)
I DO NOT KNOW
NO (false)
2. Identification of virulence factors

Identification of *S. aureus* delta-toxin

15 of 95 isolates from bone and joint infection: exhibited no delta-toxin production → chronic infection


Valour F Pclin Micobiol Infect 2015
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3. Typing

Q14. If all species are present in the database, is it possible to discriminate with MALDI-TOF closely related species such as Members of the *Acinetobacter baumannii* group?

**YES (correct)**

**I DO NOT KNOW**

**NO (false)**
2. Routine identification of bacterial isolate

**Discrimination between closely-related species**

Identification of genomic species from the *Acinetobacter baumannii* group

*A. nosocomialis* not in the commercial database!

Espinal P. et al.
1. What is Maldi-tof?
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7. Conclusions
Q15. Is it possible to see specific ertapenem peaks if you perform a MALDI-TOF analysis on a sample containing about 50 mg/ml of ertapenem?

YES (correct)
I DO NOT KNOW
NO (false)
4. Detection of carbapenemases

**Loss of peaks**

- Loss of peaks at 476 Da, 498 Da and 521 Da

Q16. Is MALDI-TOF better than PCR-based tests such as the PCR-Check MDR Carba to detect carbapenemases?

YES (correct)
I DO NOT KNOW
NO (false)
4. Detection of carabapenemases

**Modified test**

using a 10 microg ertapenem disk; only 1 hour incubation

<table>
<thead>
<tr>
<th>Diagnostic approach</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenotypic detection (Modified Hodge test)</td>
<td>90.5% (19/21)</td>
<td>86% (24/28)</td>
</tr>
<tr>
<td>Phenotypic detection (IP/IPI)*</td>
<td>54.5% (6/11)</td>
<td>92.1% (35/38)</td>
</tr>
<tr>
<td>PCR-Check MDR Carba</td>
<td>90.5% (19/21)</td>
<td>100% (28/28)</td>
</tr>
<tr>
<td>Microarray</td>
<td>90.5% (19/21)</td>
<td>100% (28/28)</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>100% (21/21)</td>
<td>100% (28/28)</td>
</tr>
<tr>
<td>MS/MS</td>
<td>100% (21/21)</td>
<td>100% (28/28)</td>
</tr>
<tr>
<td>Phenotypic detection of metallo-β-lactamases (VIM, IMP, NDM)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Detection of carabapenemases

1. What is Maldi-tof ?
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5. Quality issues

Q17. In a diagnostic laboratory, the maintenance of the Bruker MALDI-TOF may be done only once each two years? (since Bruker mass spectrometry are so robust …)

YES (correct)
I DO NOT KNOW
NO (false)
5. Quality issues

**Routine maintenance**

\[ \geq 1x/3 \text{ months in Lausanne} \ (\sim 5 \text{ run/days}) \]
Q18. Regular maintenance are needed among others to remove residues left following the carbonisation of the bacterial proteins embedded in the matrix?

YES (correct)
I DO NOT KNOW
NO (false)
7. Quality control: maintenance

**Problems:**

1. vacuum failure  
   - dust on plastic joints  
   - loss of etancheity of the plastic joints

2. carbonisation of bacteria (laser may be soiled)
5. Quality issues

Q19. The BTS calibration control from Bruker includes among other the myoglobin?

YES (correct)

I DO NOT KNOW

NO (false)
7. Quality control: maintenance

**Calibration «BTS» control**

**E. coli**
(RL36, RS32, RS34, RS33, RL29, RS19)

**RNAse A**
**Myoglobin**
Q20. When we observe the presence of residual proteins, not related due to inadequate cleaning of re-usable MALDI microplates, one solution may be to use disposable microplates?

YES (correct)
I DO NOT KNOW
NO (false)
5. Quality issues

Yes, disposable MALDI microplates

Bruker disposable plate

Hudson disposable plate

www.maldiplate.com
5. Quality issues

Q21. Is the Bruker score reflecting the size of the peaks?

YES (correct)
I DO NOT KNOW
NO (false)
Reproducibility

*S. aureus*

10^6 bacteria/well

Scores always 2.3-2.5

Do not only rely on scores
- check spectra
- use other strains, other algorithms,…

Croxatto, Prod’hom & Greub, FEMS Microbiology Reviews 2011
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6. Costs issues

Q22. Would you save money if you move towards MALDI-TOF based identification?

YES (correct)
I DO NOT KNOW
NO (false)
8. Conclusions

Q5. Would you save money if you move towards MALDI-TOF based identification?

**YES (correct)**

assuming cost of technician time of 1.2 €/min

with MALDI-TOF* without MALDI-TOF

mean cost 4.03€ 9.43€
annual cost 71’921€ 168’094€

→ 2.34 fold reduction

Including acquisition (5 year amortization) & maintenance

132’521€ 168’094€

→ 1.27 fold reduction

*identification by MALDI-TOF and when needed additional approaches

Heininger, Prod’hom & Greub, submitted
6. Costs issues

Q5. Even if my lab only perform 4000 identification per year?

YES (correct)
I DO NOT KNOW
NO (false)
Q22. Even if my lab only perform 4000 identification per year?

NO

Mean traditional identification cost
MALDI-TOF cost with a 5 years amortization
MALDI-TOF cost with a 10 years amortization

Already cost saving when performing 5’000 to 7’000 tests per year
1. What is Maldi-tof? 
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7. Conclusions
Q23. What you learned during this ESGMD postgraduate course will it be useful in your daily work?

YES (correct)
I DO NOT KNOW
NO (false)
7. Conclusions

Q24. Would you like to have a ESGMD postgraduate course with practicals?

YES (correct)
I DO NOT KNOW
NO (false)
Thank you

13-15 March 2017, Basel

4-7 September 2018, Lausanne
Practicals
Social program (including the running club)
A similar meeting report will be prepared by Jacob Gilad-Moran, John Rossen, Adrian Egli and me …..
Many thanks to Birke Mebold, Adrian Egli, Thomas Greif, Jacob Gilad-Moran, John Rossen, all speakers and all of you...