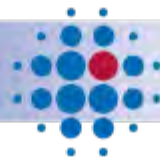


MALDI-TOF mass spectrometry for typing

Axel Karger and Lutz Geue

Friedrich-Loeffler-Institut
Federal Research Institute for Animal Health
Insel Riems and Jena

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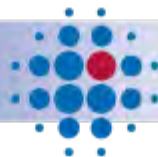
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Overview

- About whole cell MALDI-MS
- Serotype determination of Enterohemorrhagic E. coli (EHEC)
- Discrimination of Burkholderia mallei/ B. pseudomallei
- Characterisation of Brucella species and biovars
- Strategies for the discrimination of closely related organisms

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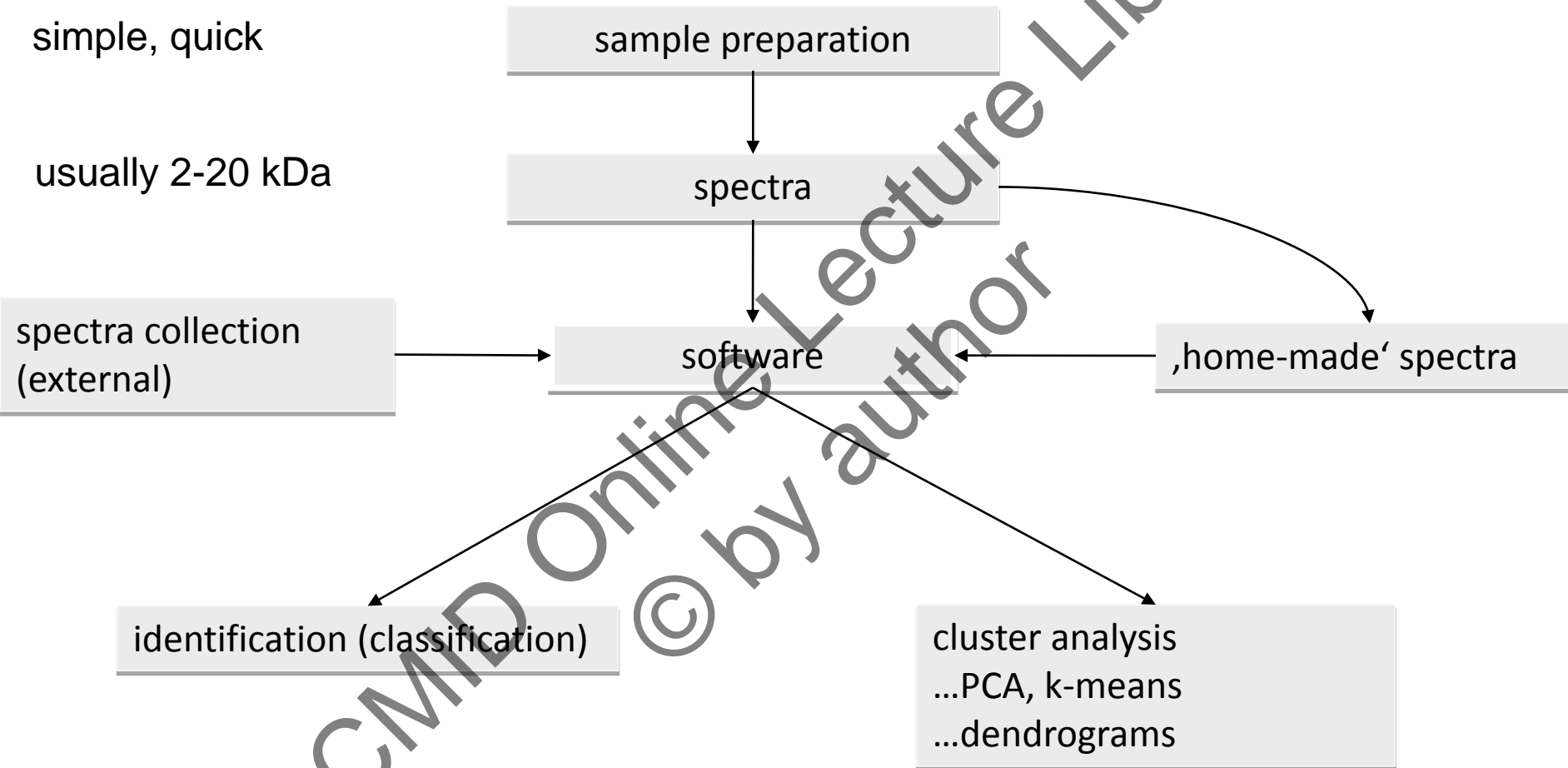
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Typical workflow for MALDI-typing experiments

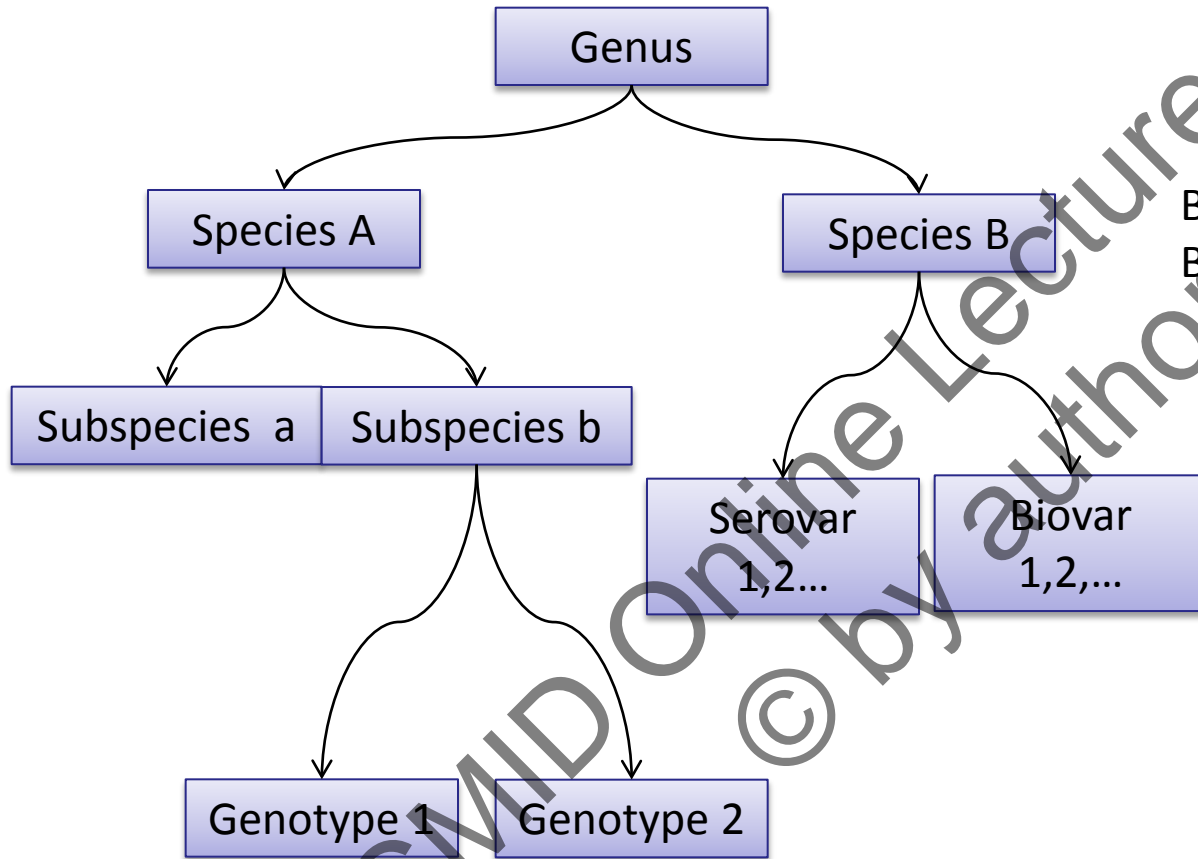
simple, quick

usually 2-20 kDa



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To which taxonomic level will MALDI-typing work?



Burkholderia mallei/pseudomallei
Brucella species

Brucella suis biovars
EHEC serotypes

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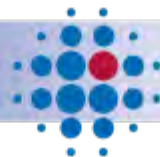
Strategies for the discrimination of closely related organisms by MALDI-typing

Main problem:

Spectra are very similar, thus classification may be difficult.

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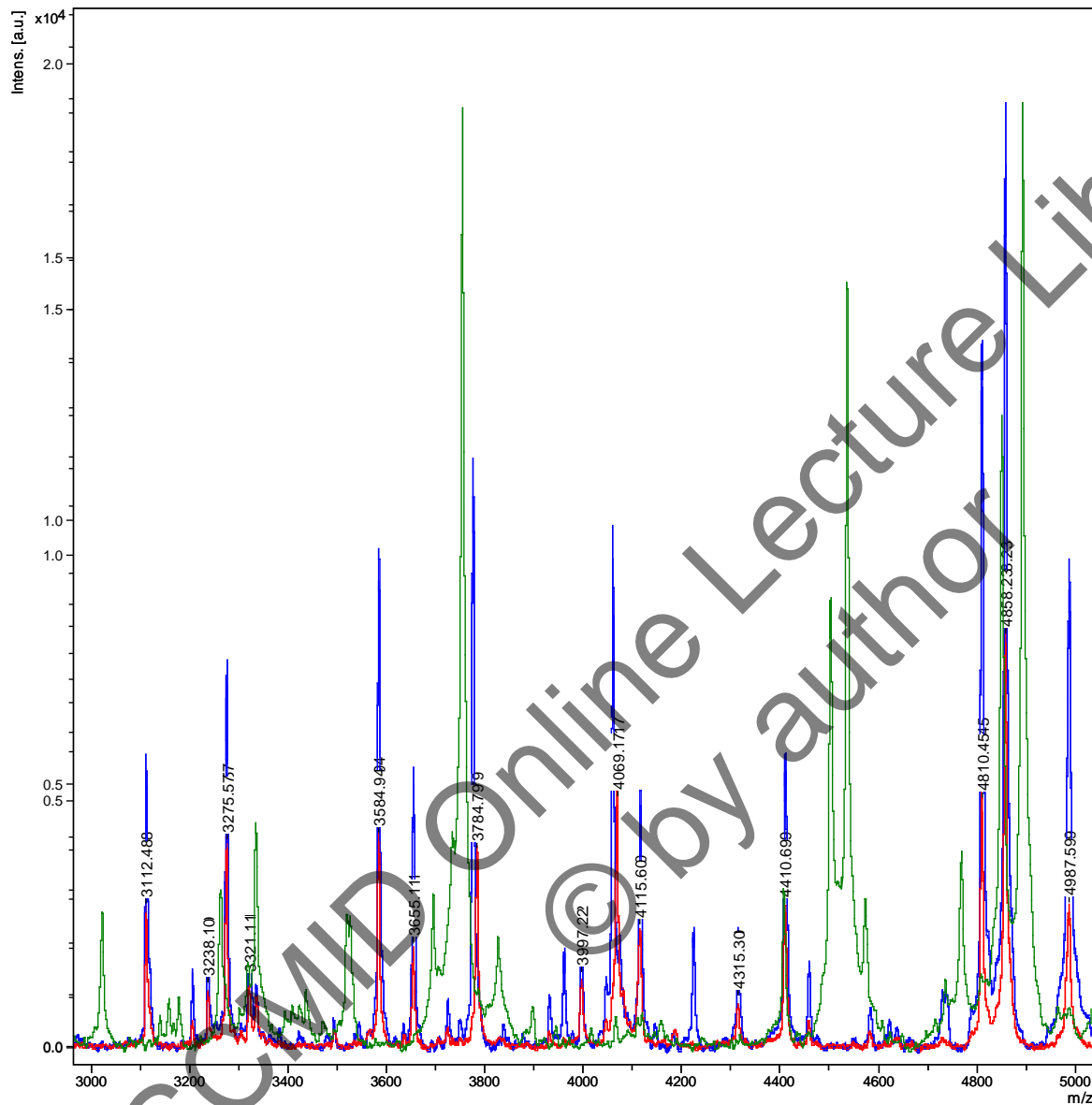


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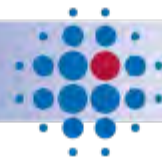


Burkholderia mallei
B. pseudomallei
Brucella abortus

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Strategies for the discrimination of closely related organisms by MALDI-typing

Main problem:

Spectra are very similar, thus classification may be difficult.

Possible strategies:

Bioinformatics/statistics:

- Data reduction by refinement of peak selection
- Selection of classifiers
- Identification of taxon-specific markers

...

Experimental:

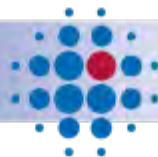
- Proteolytic digestion
 - Extraction protocols
 - Typing of PCR products
- ...

Serotype determination of EHEC

- Produce Shiga-toxin
- Occur in certain serotypes
- Reservoir in cattle
- Can cause hemolytic-uremic syndrome (HUS) in humans
- EHEC O104:H4 outbreak in Germany spring/summer 2011

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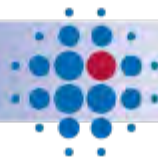
Serotype determination of EHEC

Samples collected in a monitoring program in cattle farms:

- 94 EHEC isolates representing three O-serotypes
- O165:H25 (n = 30)
- O26:H11/H32 (n = 46)
- O156:H25 (n = 18)
- analysis by Restriction Fragment Length Polymorphism
- farm-specific RFLP-clusters within the serotypes

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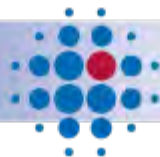
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Serotype determination of EHEC

Can the three O-types and the farm-specific genetical clusters within the serotypes be reproduced by MALDI-typing ?

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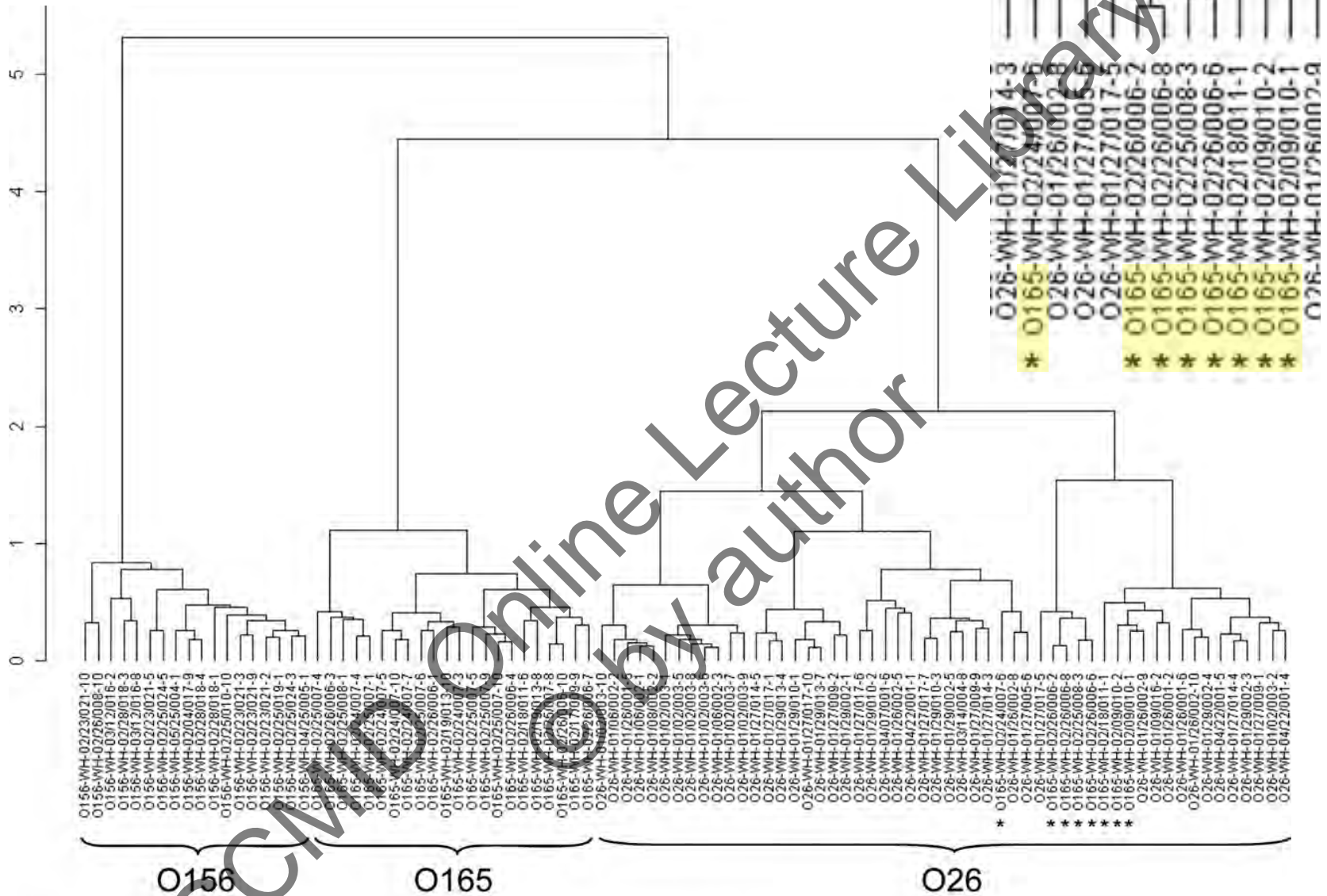


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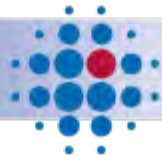
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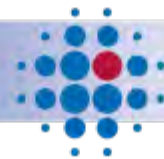
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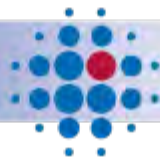
Serotype determination of EHEC

Is the spectrum-based separation sufficient for classification ?

- Standard software failed
- Classification by comparison with ,prototype' spectra

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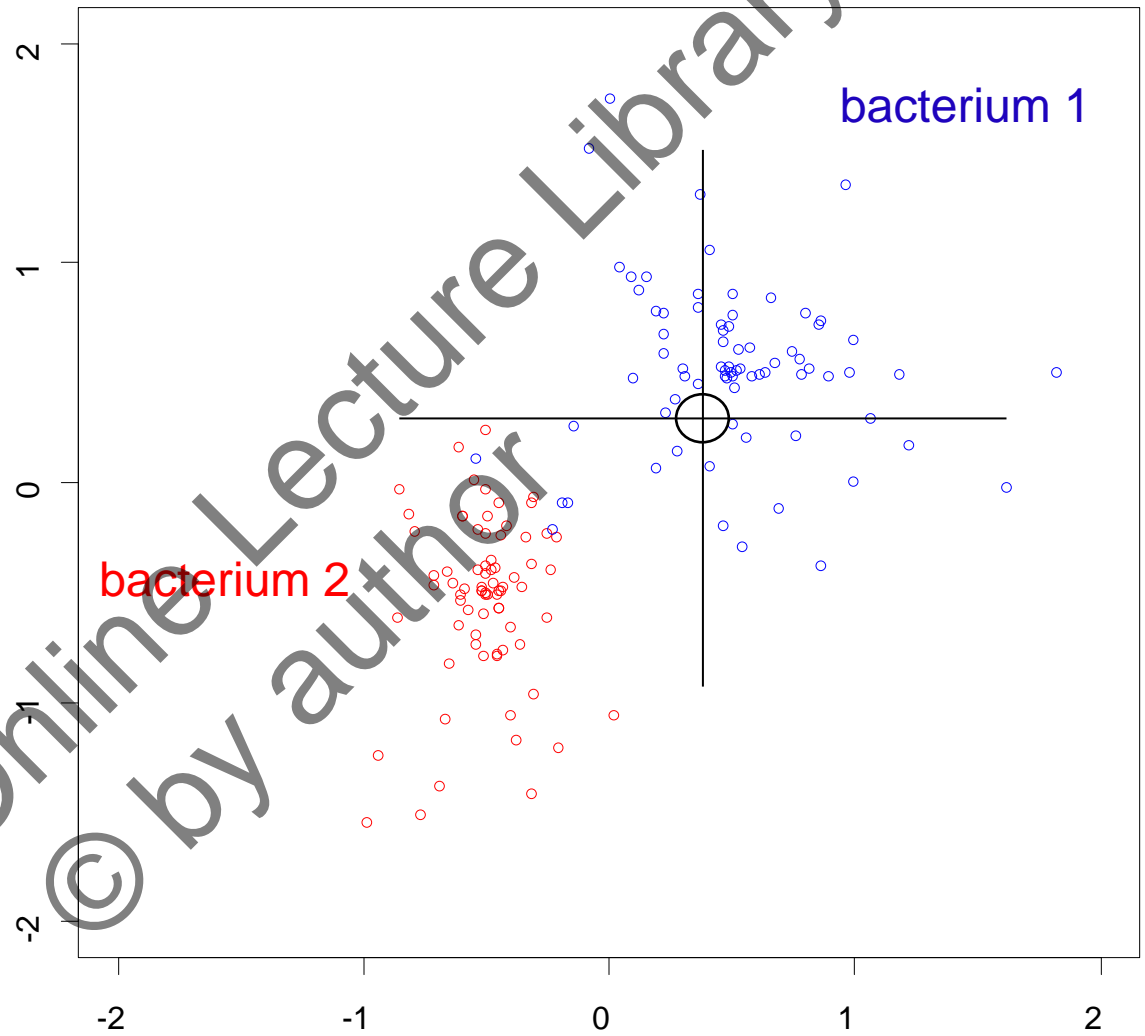
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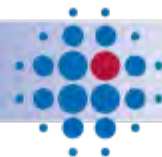
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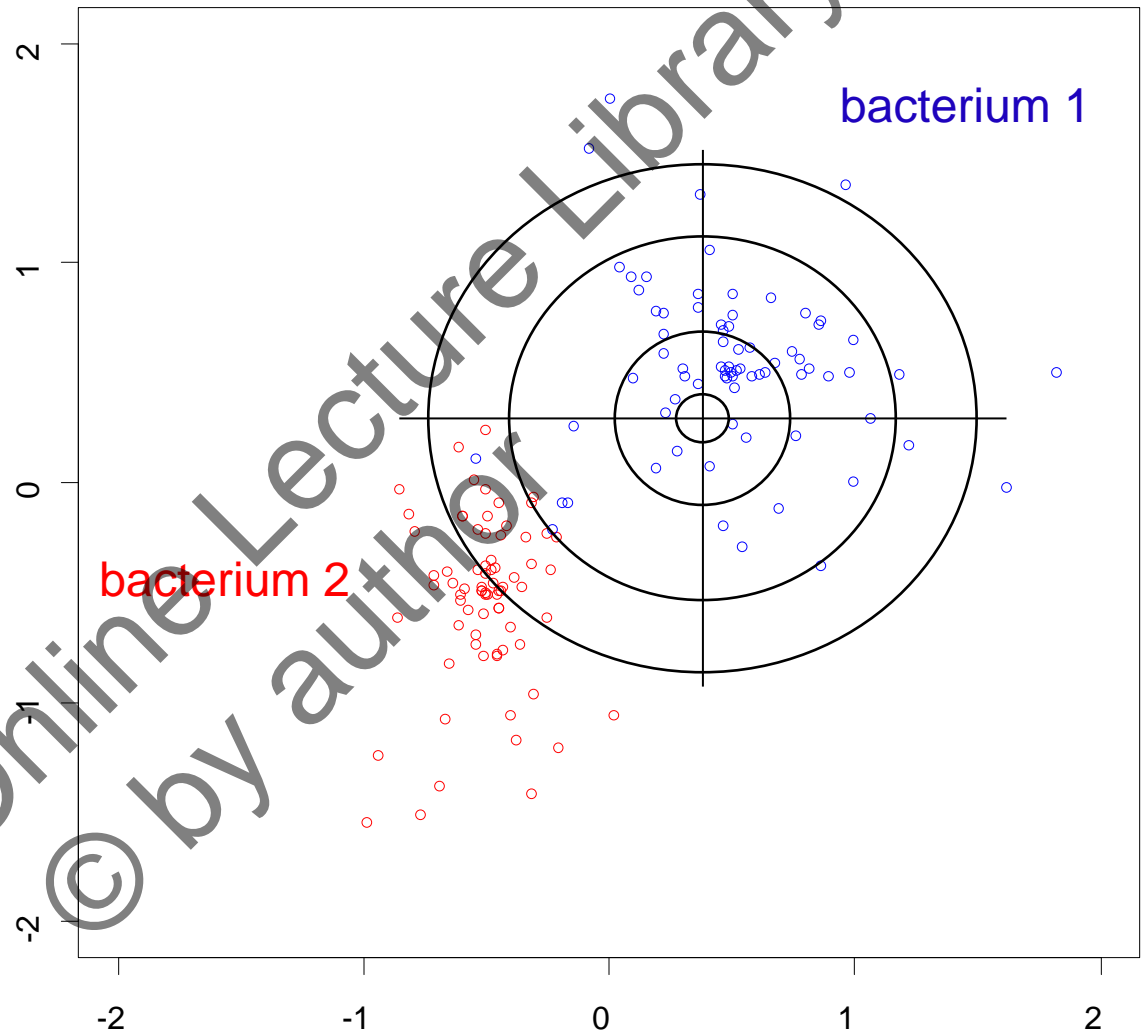


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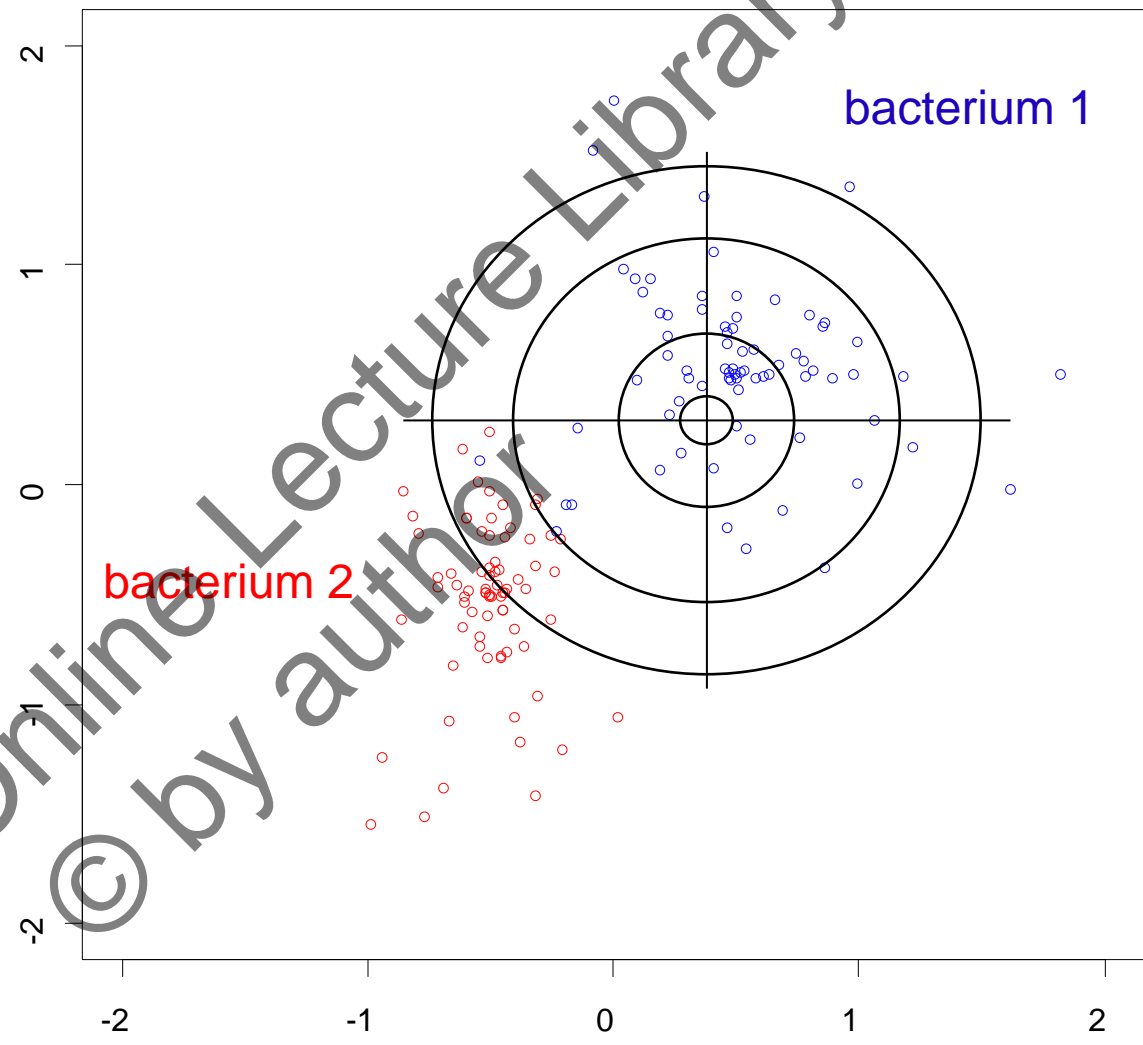
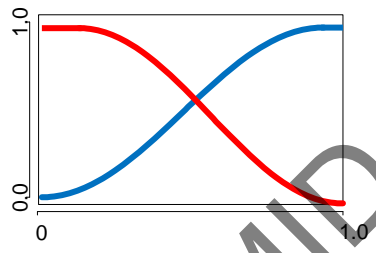
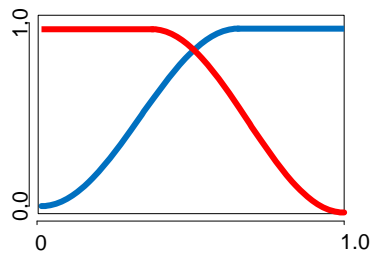
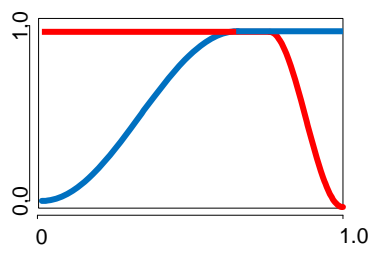
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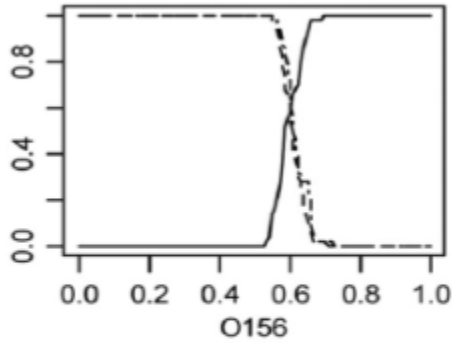


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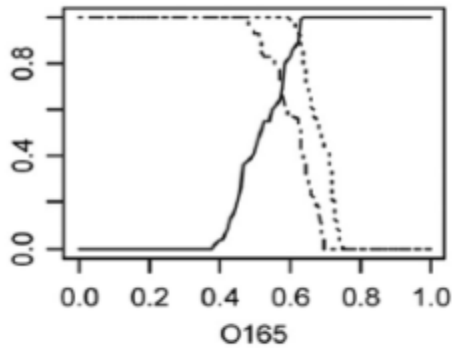


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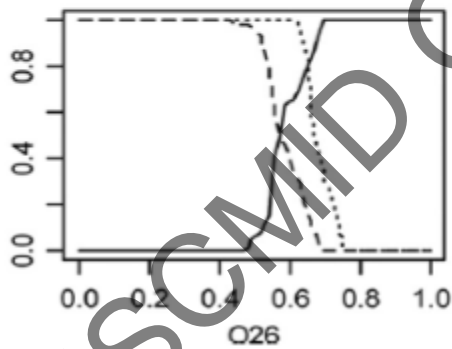
O156



O165



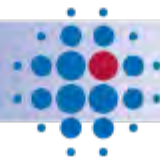
O26



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Serotype determination of EHEC

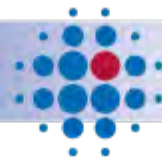
Data reduction strategy:

- gradually remove masses from the prototype spectra which do not contribute to group separation

Two parameters were varied systematically:

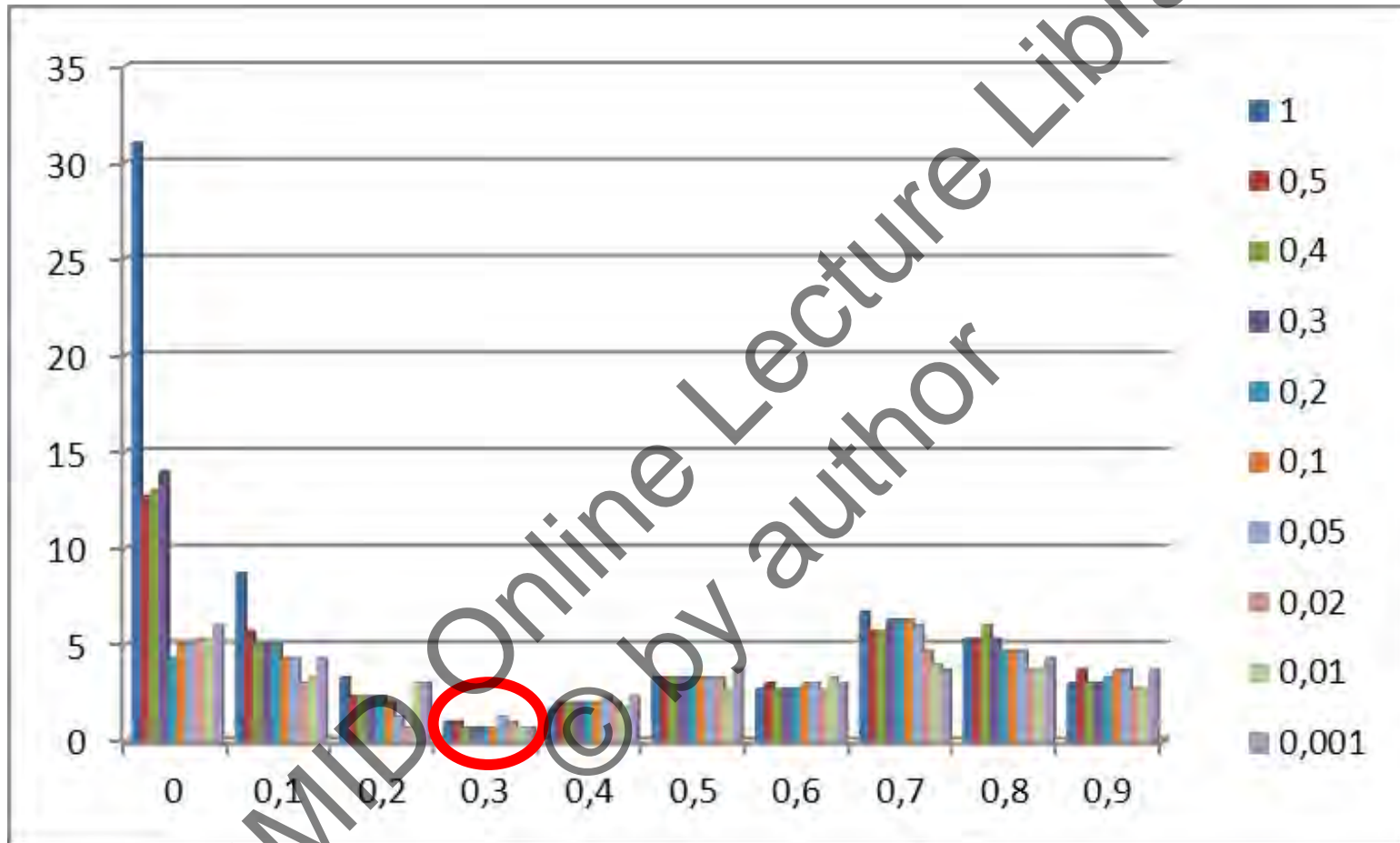
- minimal frequency of a mass in a serotype group
- significance level of Fisher's t-test for every mass.

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Misidentifications in %

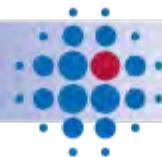
Fisher's t-test
significance level



minimal frequency

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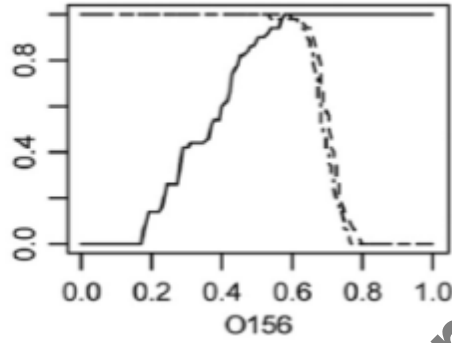
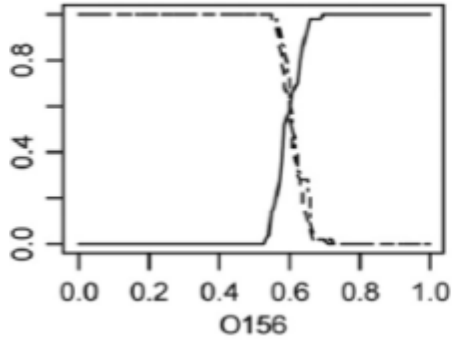
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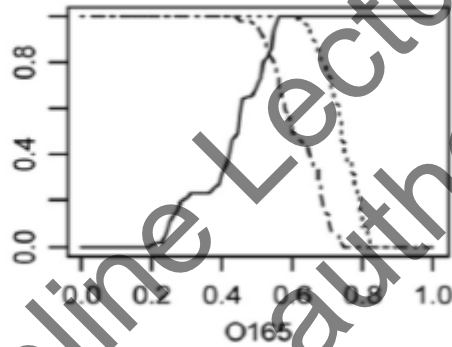
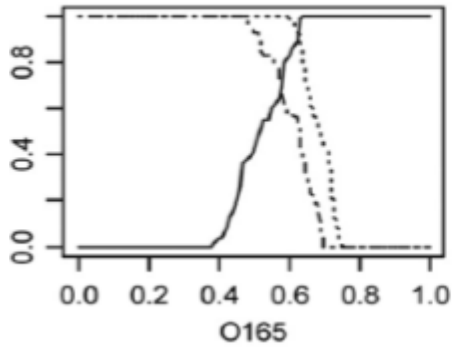
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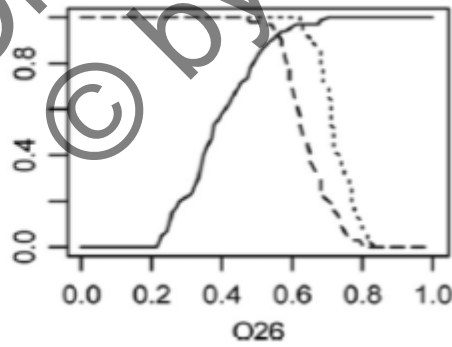
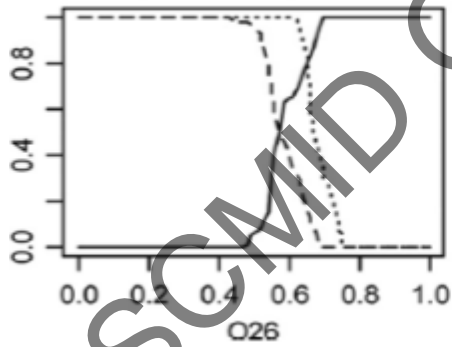
O156



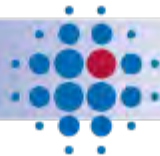
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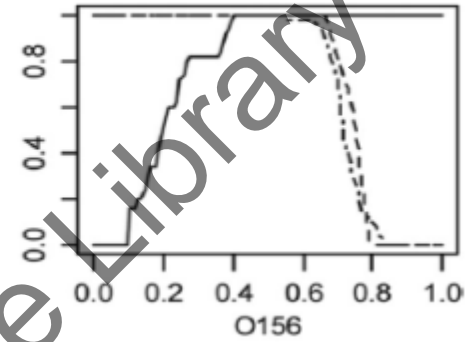
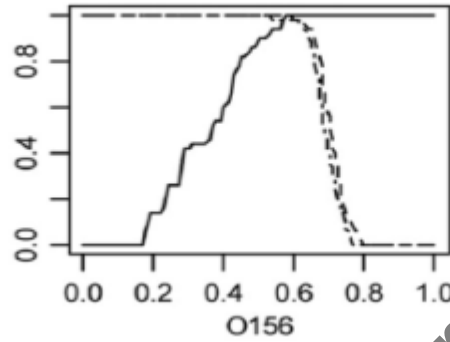
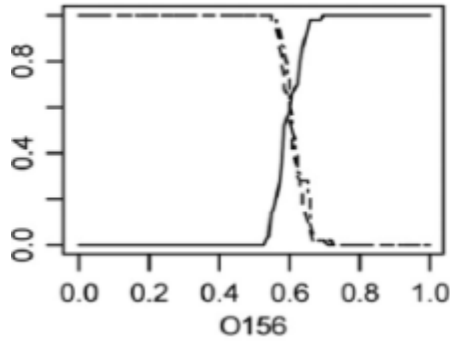
O26



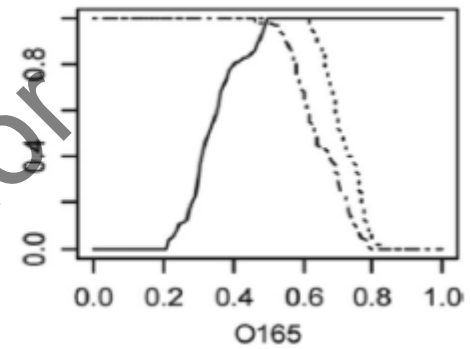
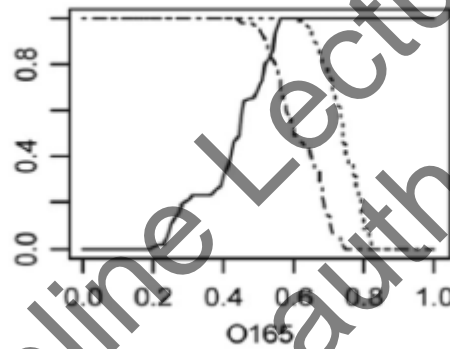
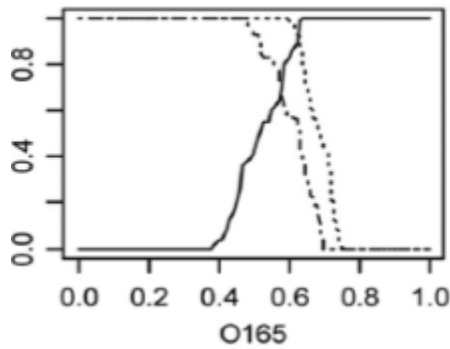
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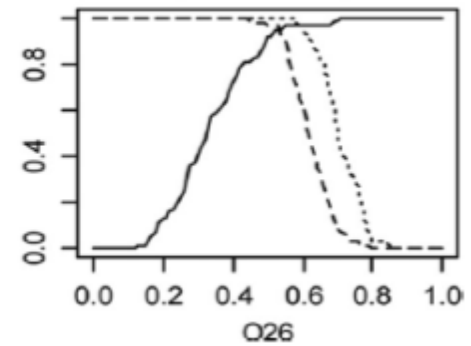
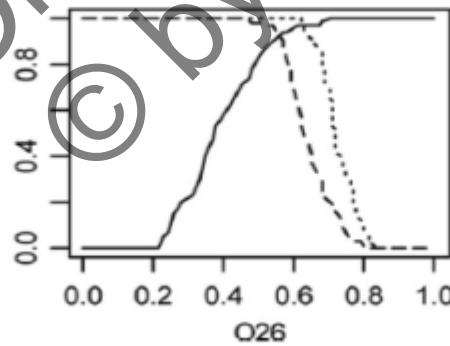
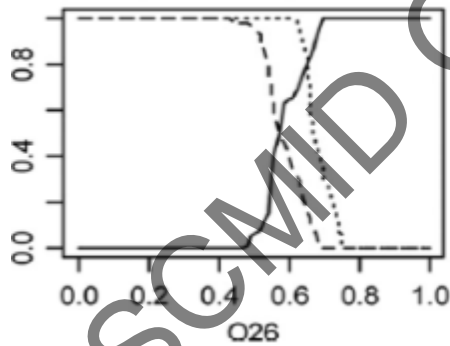
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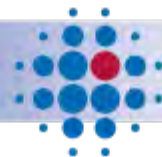
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O26



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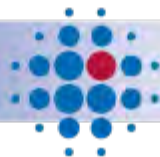


Serotype determination of EHEC

Can farm-specific genetic clusters within EHEC O26 be reproduced by MALDI-typing?

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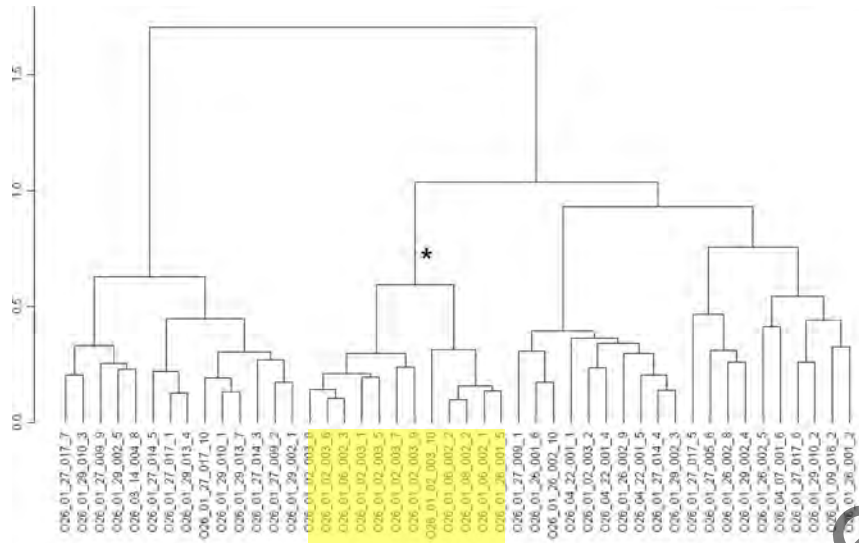


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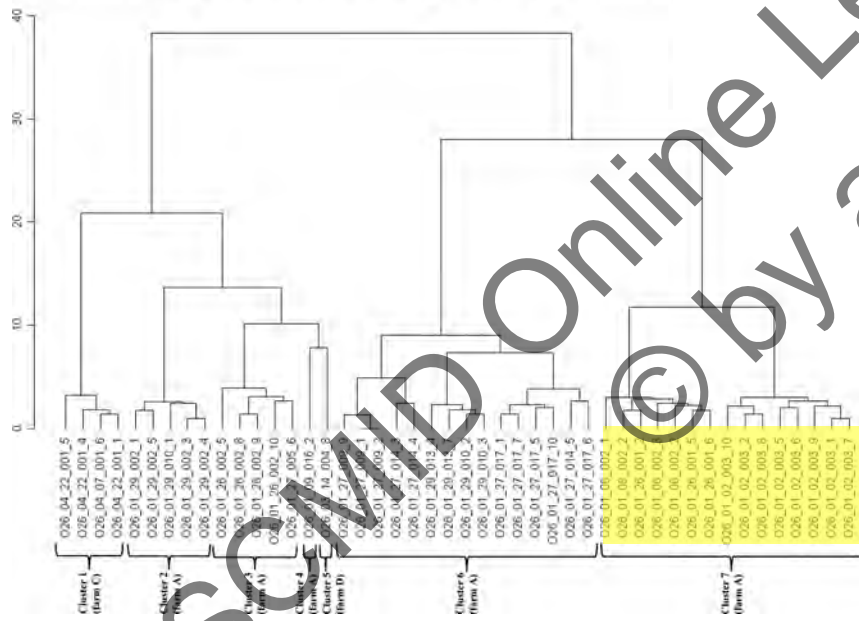
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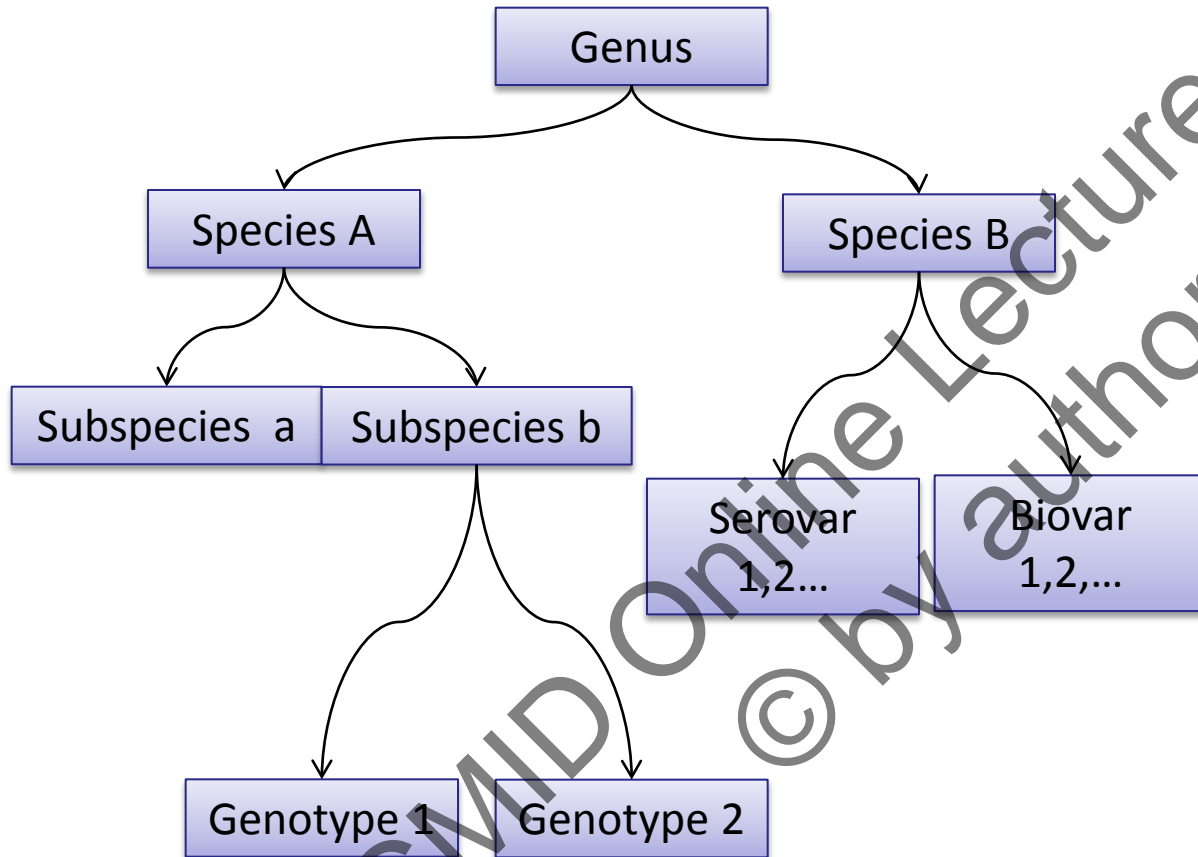
MALDI spectra



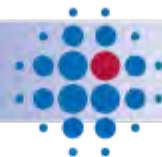
Restriction Fragment Length Polymorphism analysis

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E. coli



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Differentiation of *Burkholderia mallei*/*B. pseudomallei*

- Two different species but closely related
- Causative agent of glanders and melioidosis

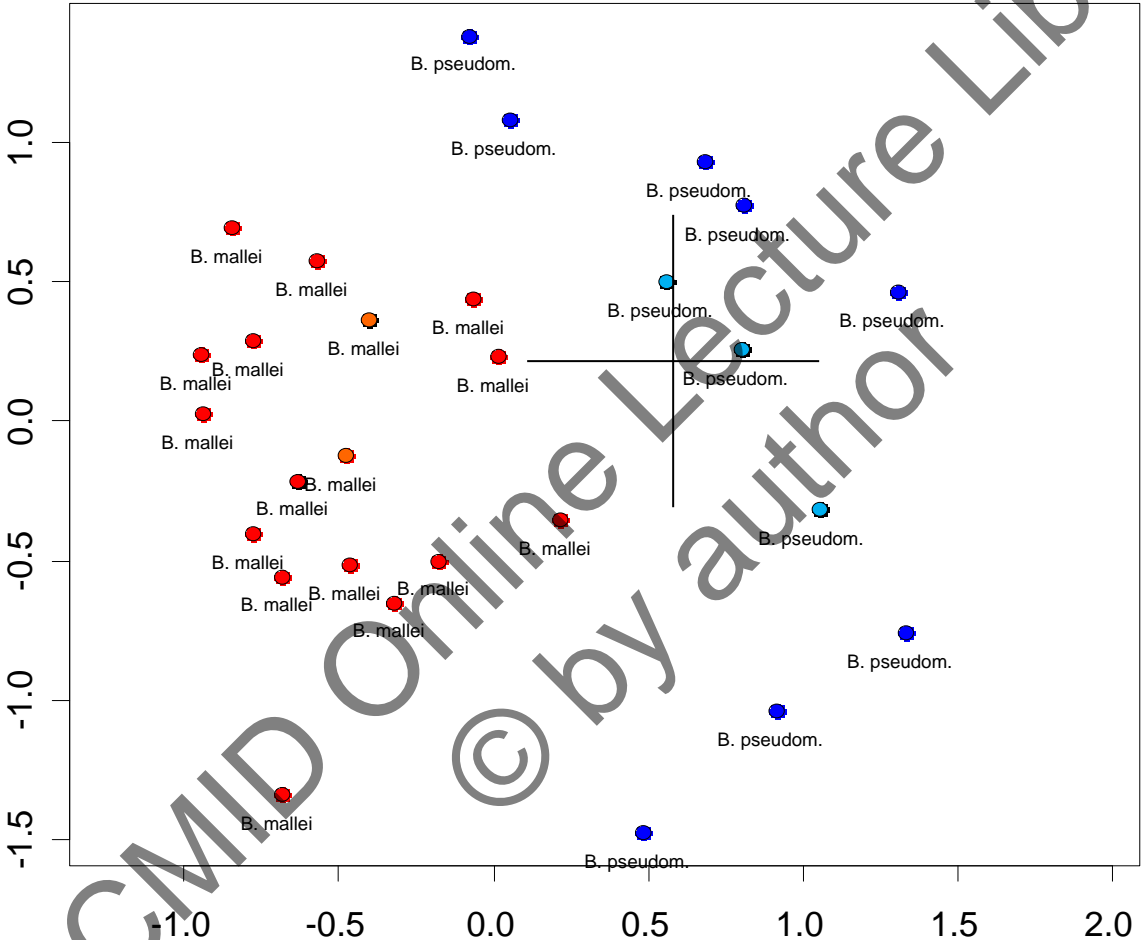
Reference spectrum collection of

B. mallei (n=17)

B. pseudomallei (n=10)

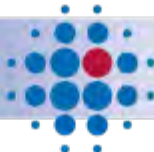
- MALDI Biotyper scores were very close in some cases
- Construction of prototype spectra for every species failed

Distance relations between *B. mallei* / *B. pseudomallei*



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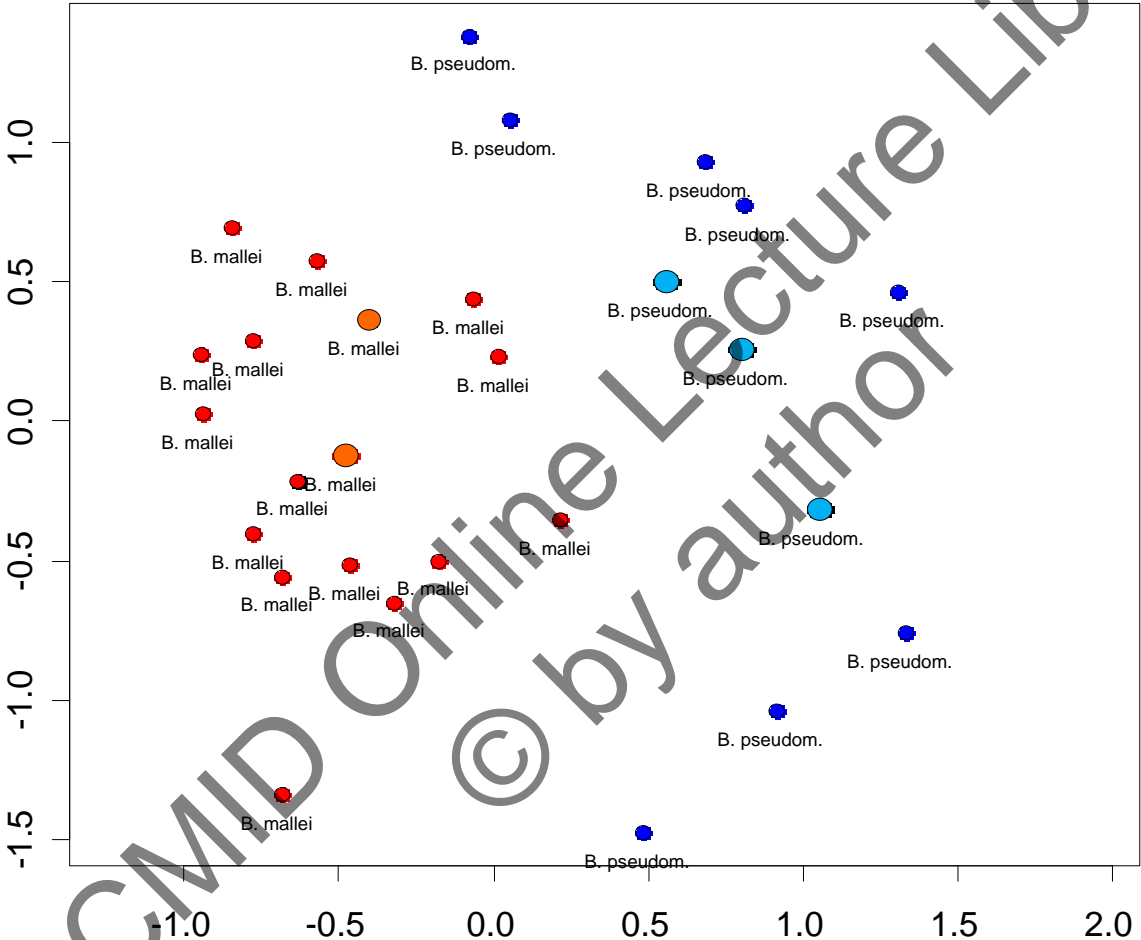
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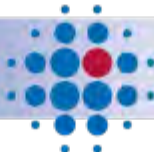
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Distance relations between *B. mallei* / *B. pseudomallei*



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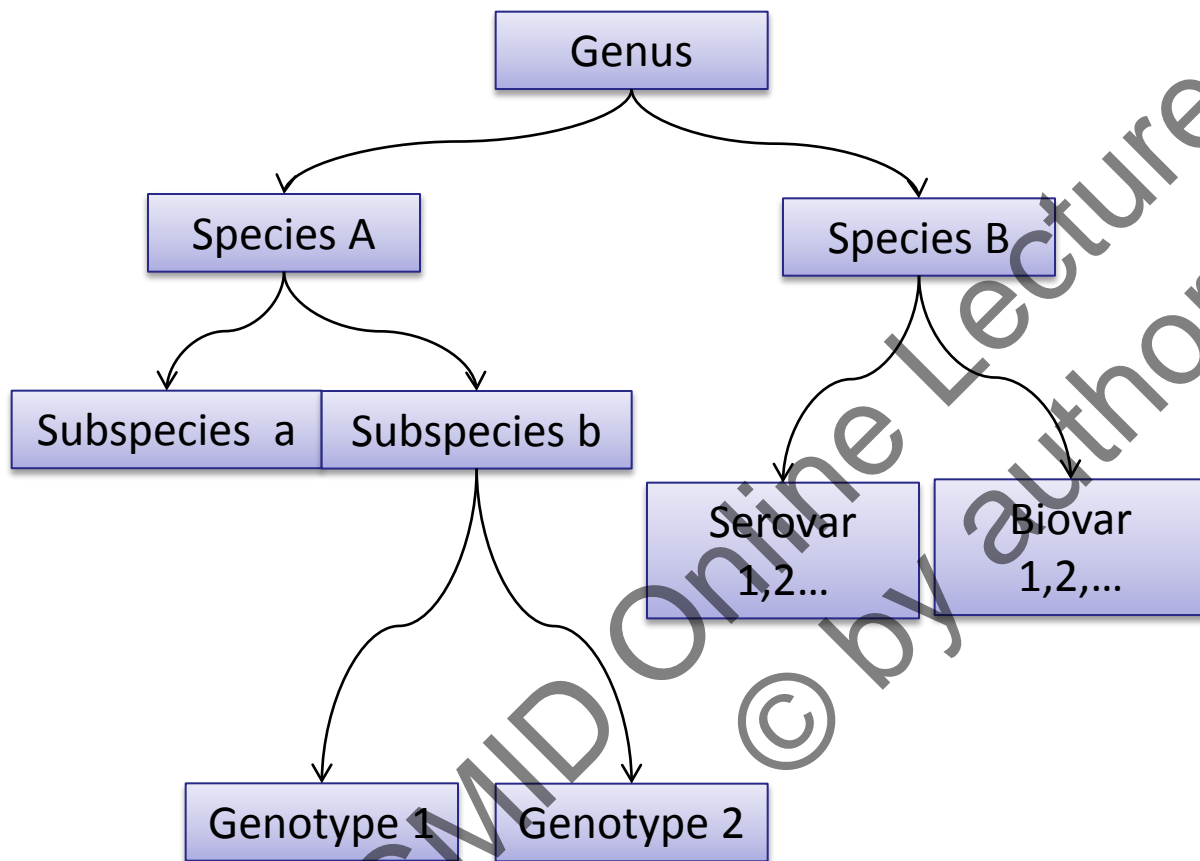
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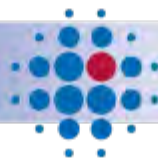
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Burkholderia



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Separation of Brucella species and biovars

Poster 2340

Material:

Reference spectra from a set of 44 isolates representing

B. abortus

B. canis

B. ceti

B. inopinata

B. melitensis

B. microti

B. ovis

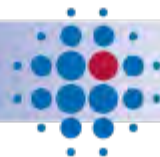
B. pinnipedialis

B. suis

Extracts were prepared and spectra taken in two independent laboratories with different MS equipment.

Additionally 2 sets of field isolates (n=67 and 82), analysed in one lab each.

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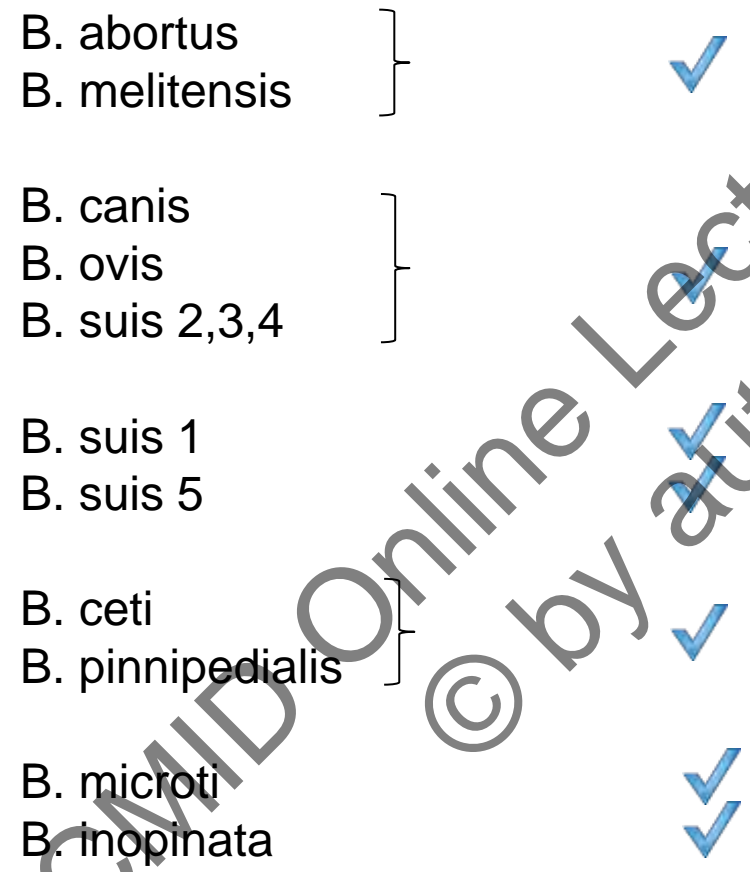
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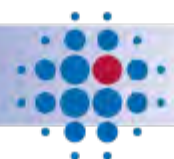
Separation of Brucella species and biovars

MALDI Biotyper



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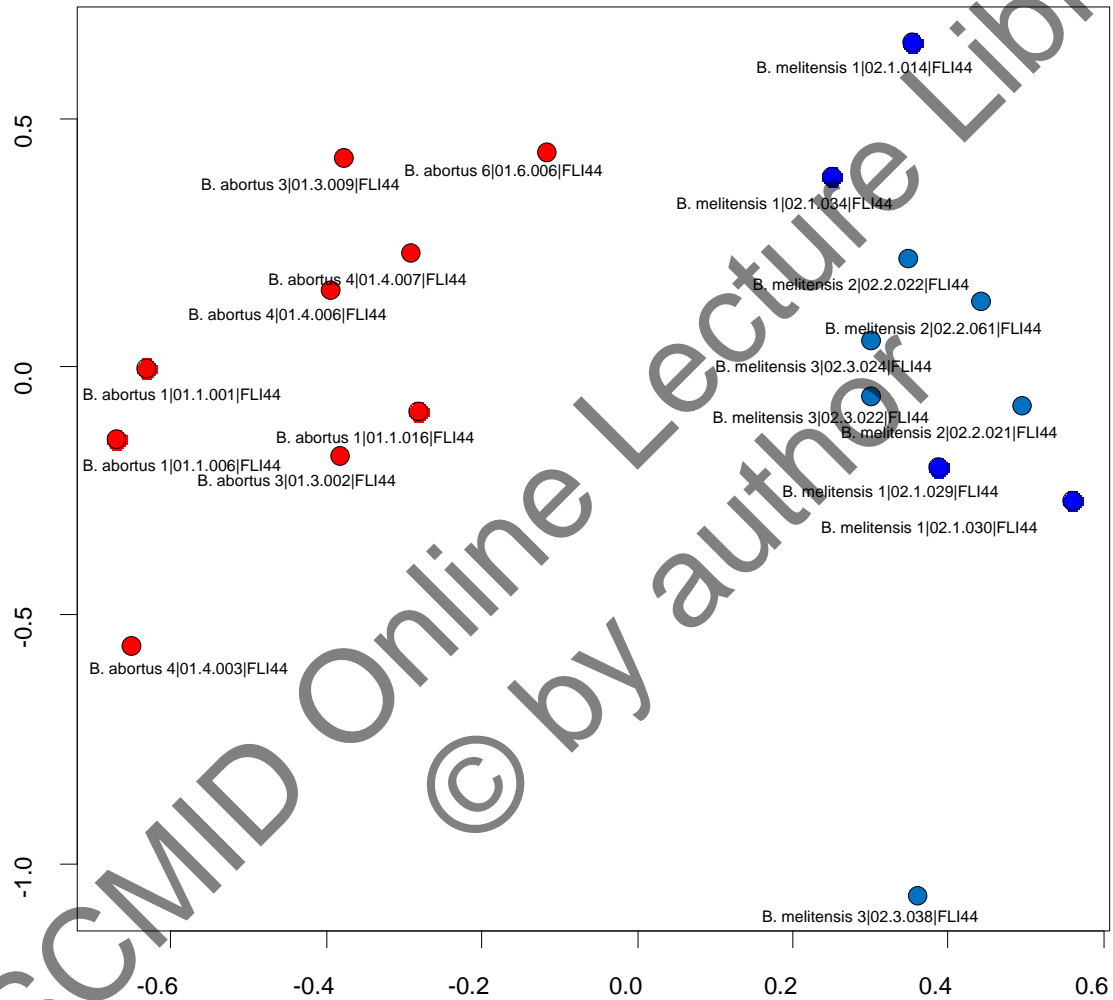
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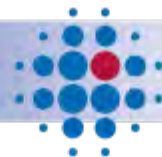
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Distance relations between *B. abortus* / *B. melitensis*



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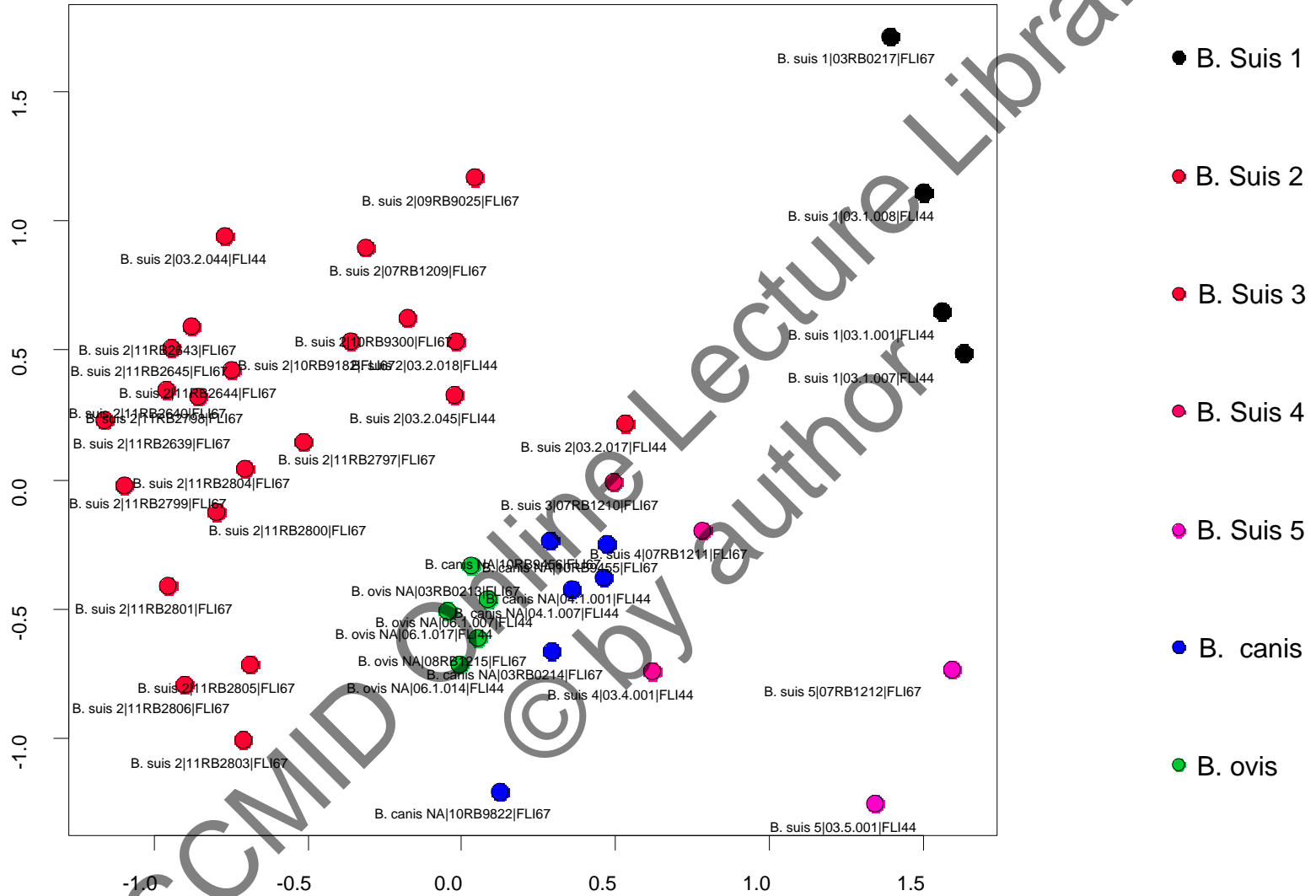




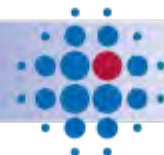
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Distance relations between B. suis / B. canis / B. ovis



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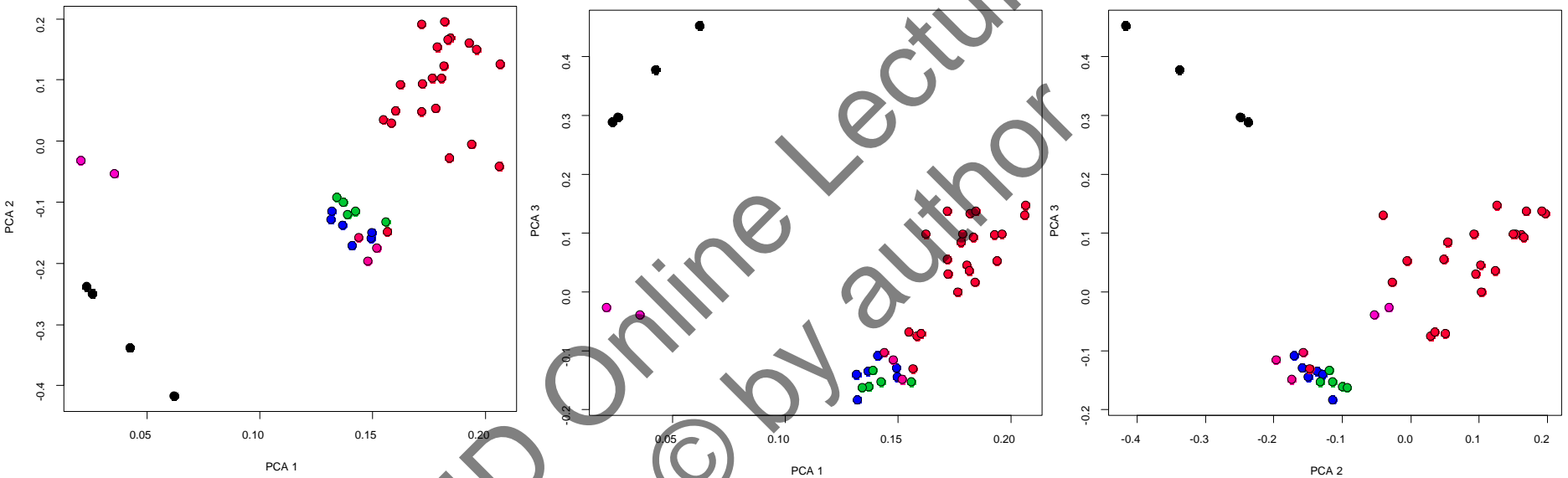


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Principal component analysis of *B. suis* / *B. canis* / *B. ovis*

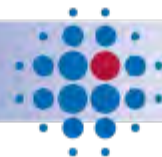
● B. Suis 1 ● B. Suis 2 ● B. Suis 3 ● B. Suis 4 ● B. Suis 5 ● B. canis ● B. ovis



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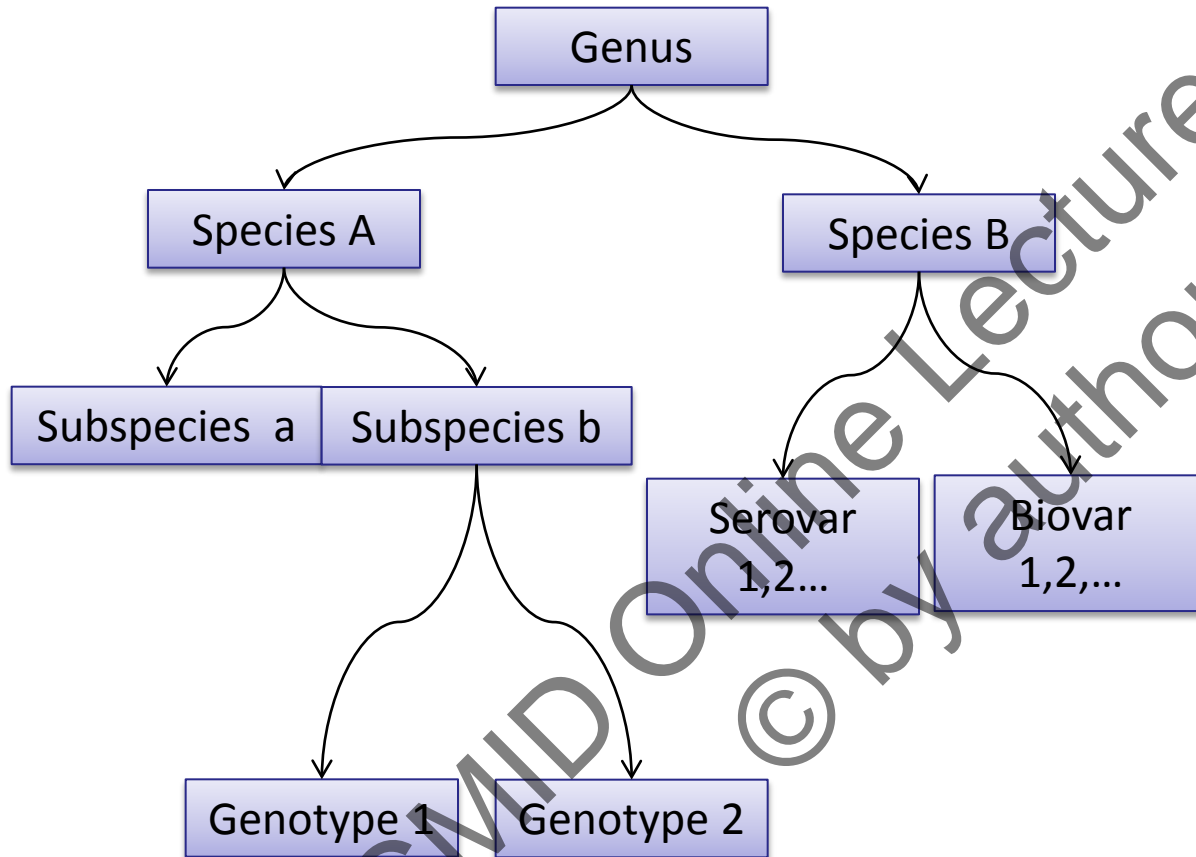
100 JAHRE

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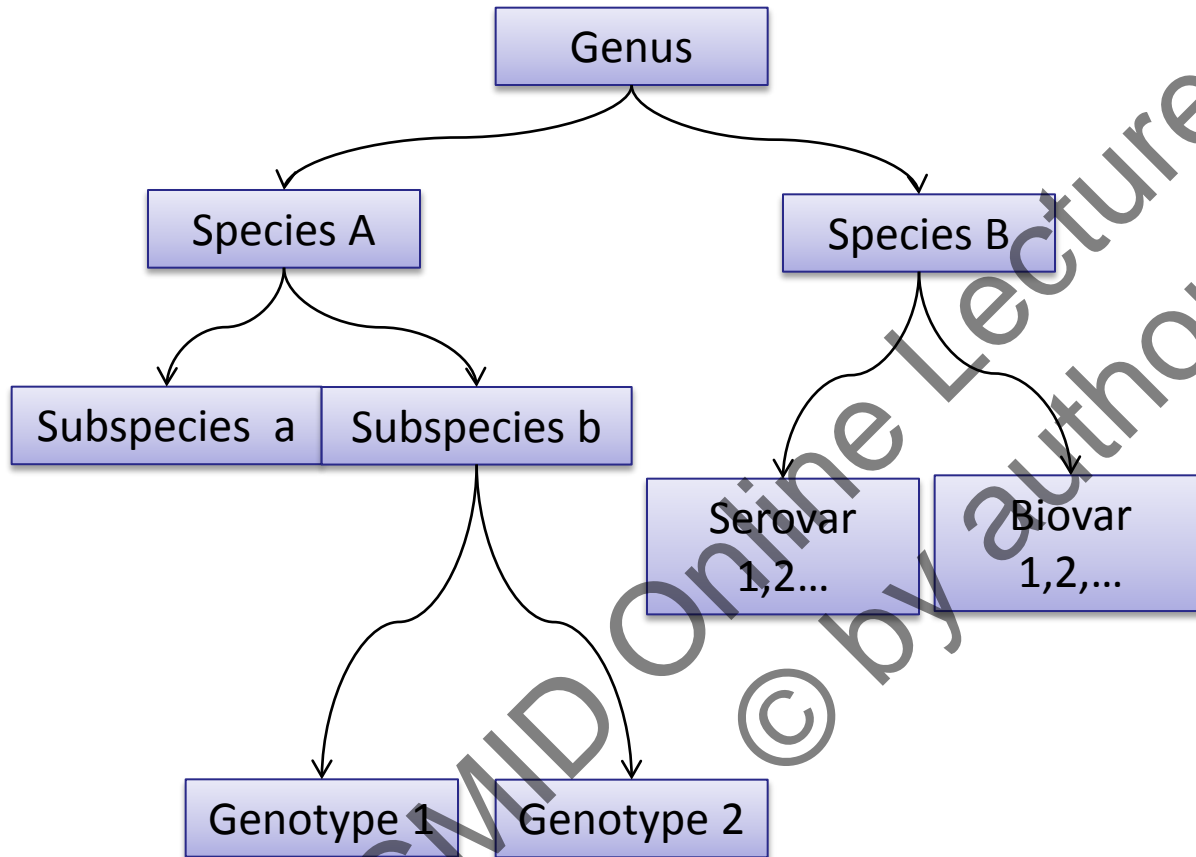
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Brucella

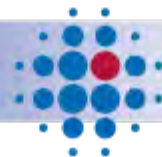


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Brucella



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Conclusions I (Bacteria)

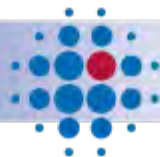
- Discrimination of some *Brucella* species and biovars is possible with MALDI Biotyper software. A mass-based decision tree is required for the resolution of all species. Distance between *B. suis* 1 and 2 is larger than between *B. suis* 2 and *B. canis*.
B. canis can hardly be discriminated from *B. suis* 3 and 4.
- Concept of prototype spectra was successfully applied to the serotype determination of three serotypes of EHEC
- Discrimination of *Burkholderia mallei*/*B. pseudomallei* with MALDI Biotyper is possible with a dedicated reference spectrum set

Conclusions II (technical)

- Keep protocols as simple and stable as possible
- Be aware of artifacts and confounders
- Cross-check with a second laboratory
- Carefully select reference spectra. Less can be more.

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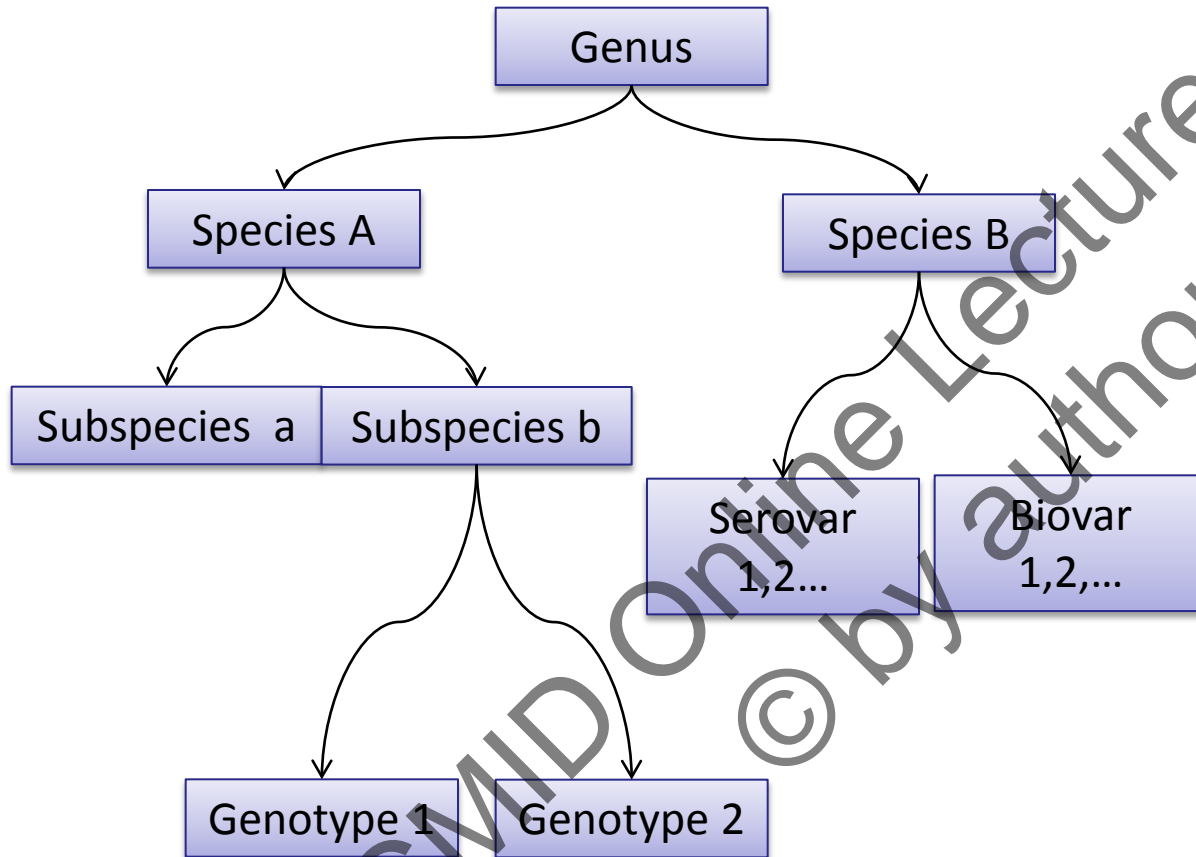
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To which taxonomic level will MALDI-typing work?



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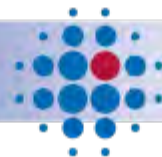
Bruker Bremen

M. Kostrzewa, T. Maier
M. Timke



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