Identification of *Brucella* spp. isolates by MALDI-TOF Mass Spectrometry

Sali M.*º, De Maio F.*º, Tarantino M.***, Garofolo G.**, Pasquali P.***, Petrucci P.***, Sanguinetti M.*, Di Giannatale E.**, Tittarelli M.** and Adone R.***

*Università Cattolica del Sacro Cuore, Rome, Italy **Istituto Zooprofilattico Sperimentale G. Caporale, Teramo, Italy ***Istituto Superiore di Sanità, Rome, Italy.

ºThese authors contributed equally to this work

Background

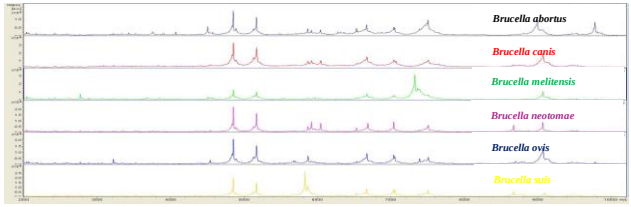
Six species, some of which subdivided into biovars, are traditionally assigned to the *Brucella* genus: *B. melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, *B. neotomae*. Recently, *B. pinnipedialis* and *B. ceti*, from marine mammals, have been added. *B. melitensis*, *B. abortus*, *B. suis* and *B. ovis*, can infect humans mainly through the consumption of contaminated dairy products. Procedures for microbiological identification and typing of *Brucella* spp. are expensive, time-consuming and require biohazard containment facilities to minimize the risk for operators.

The MALDI-TOF-MS (Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry) assay, based on the characterization of species-specific protein profiles, is a rapid, cost-effective, accurate and reproducible method for the biological samples analysis.

In this study, we assessed a new protein extraction protocol and constructed a home-made reference database to improve the efficiency of the method. To test the new library, different reference *Brucella* species and biovars, previously characterized by genotyping assay MLVA (Multiple Locus Variable-number Tandem Repeat Analysis), were used.

Materials and methods

A new, safe, protein extraction protocol easier and faster than standard method was used to obtain inactivated lysates of *Brucella* strains to assay with "*Bruker daltonik"* MALDI-TOF instrument. In order to achieve a good standardization the same culture conditions were used before MALDI-TOF preparation and analysis. New extraction protocol was used to generate specific protein profile for the new home-made database, composed by 26 different *Brucella* strains, and to identify blind-coded *Brucella* field isolates and reference strains.



Protein profiles of different Brucella species

Results

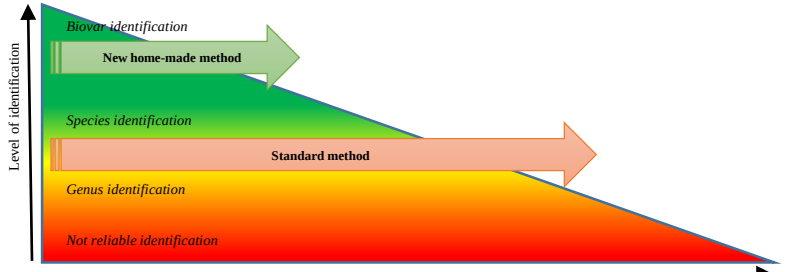
Using the new protein extraction method and the home-made reference library, the resulted always correct at the genus level. At the species level, a total of 94% bacterial samples were correctly identified. In contrast, incorrect biovar assignments resulted in 23 out of 39 *B. abortus* strains and in 4 out of 53 *B. melitensis* strains.

	MALDI Biotyper results		
Sample	**No. Correct/ total**	**No. Incorrect/ total**	**Misdiagnosis (No)**
B. melitensis	53/53		
B. abortus	36/39	3/39	*B. suis* (3)
B. suis	4/5	1/5	*B. melitensis*
B. ovis	1/1		
B. canis	0/1	1/1	*B. abortus*
B. neotomae	0/1	1/1	*B. abortus*

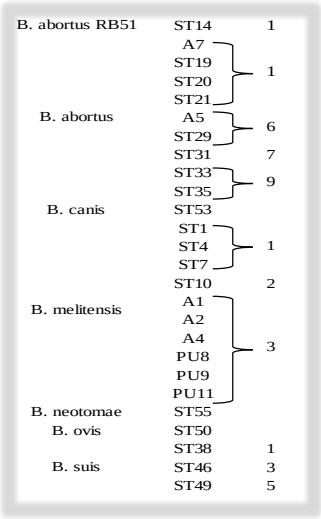
Identification at species level of blind coded and reference strains

Conclusions

Although we have used a small database, our results indicate that the MALDI-TOF-MS assay is a reliable approach to identify *Brucella* genus and species and that an higher number of different *Brucella* strains in the database could improve its discriminatory efficiency at species and biovar level. Moreover, a more complex database may allow to detect epidemiological distance between the different *Brucella* strains.

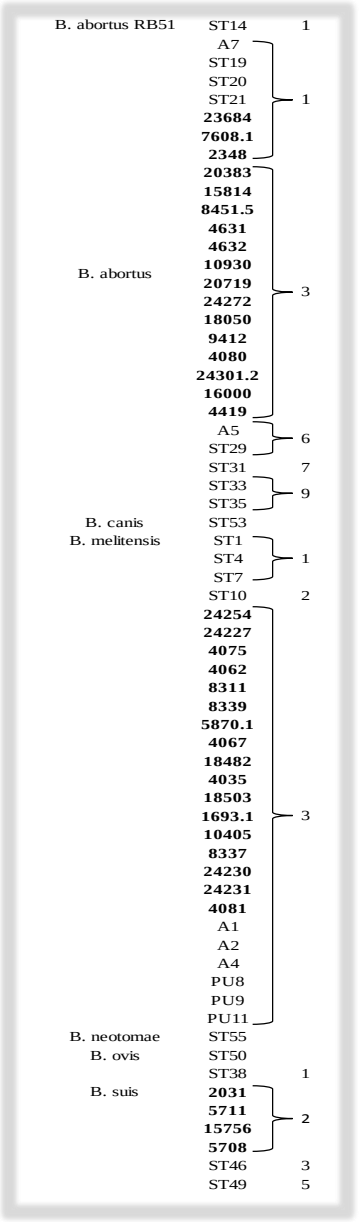


Schematic representation of discriminatory efficiency between old method and new method



Tested home-made database

Future home-made database



Tested home-made database vs new "in progress" database