

# Testing of Disinfectants for Inactivation of Vegetative Cells and Spores of *Bacillus* spp. for MALDI-TOF Mass Spectroscopy

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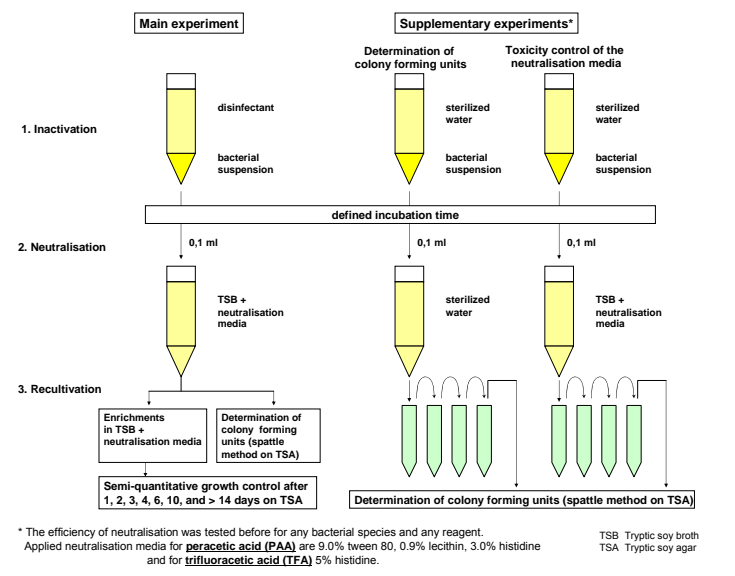
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**Introduction** The possibility of the general application of MALDI-TOF MS (matrix assisted laser desorption/ionization – time of flight mass spectroscopy) to detect BSL3 microorganisms in a B-Task Force should be assessed. This technique provides mass spectra with unique biomarker profiles on a time scale of minutes with minimal sample preparation. Drawbacks of the MALDI-TOF MS protocols suggested in the literature so far are the non-standardized conditions for sample treatment which cannot exclude survival of individual cells or spores. Based on previous experiences, methods of inactivation and sample preparation should be developed which permit safe measurements of pathogens and at the same time generate reliable mass spectra for identification of unknown samples.

Decontamination methods that reliably inactivate spores are a crucial prerequisite for safe analysis of pathogens by MALDI-TOF MS. The main challenge is that so far, no procedures are available that reliably inactivate both microbial cells and spores and at the same time preserve the necessary information for MALDI-TOF MS. Therefore, we thoroughly examined the efficiency of some chemical compounds that might be used in inactivation protocols.

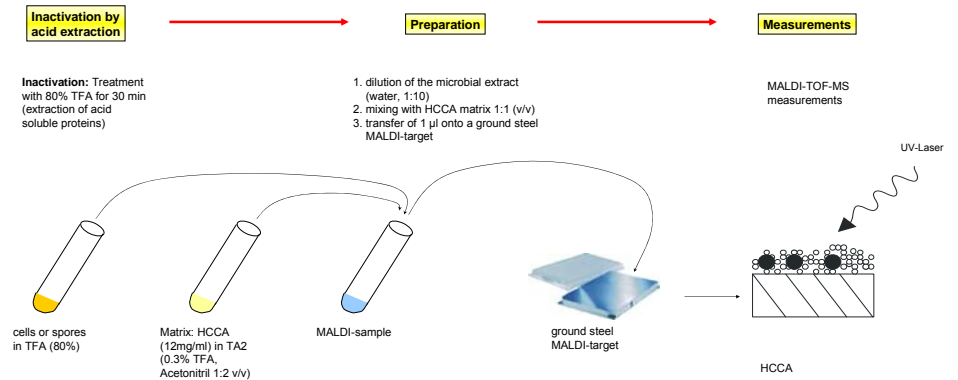
**Testing of different disinfectants** Besides the fixing solution for electron microscopy (10 % formaldehyde, 0.05 % glutaraldehyde) we tested different concentrations of peracetic acid (PAA) and trifluoroacetic acid (TFA) for spore inactivation. TFA seemed to be the most suitable chemical. A 60 % TFA solution inactivated only a part of *Bacillus* species. With a 30 min treatment of an 80 % TFA solution, spores of all *Bacillus* species tested were efficiently killed in concentrations of 10<sup>8</sup> spores/mL. However, survival of single spores from strains of *B. cereus* and *B. subtilis* was observed in enrichment cultures when spore concentrations of 10<sup>10</sup>/mL were tested.

## Test procedure for disinfectants of bacteria/spores in quantitative suspension assays



**Conclusions** Inactivation of vegetative cells and spores by TFA treatment is a reliable technique that permits a comprehensive analysis of microbial protein patterns by MALDI-TOF mass spectroscopy. **But** the killing efficiency on high concentrations of *Bacillus*-spores (> 10<sup>9</sup>/mL) using TFA (80%, 30 min) is not safe enough! Further tests are on validation: The incubation time of TFA has to be extended on spore concentrations ≥ 10<sup>9</sup>/mL and the treatment using protecting reagents has to be tested.

### Sample preparation



### Treatment of *Bacillus*-spores using TFA (80 %, 30 min)

Spores suspension >10<sup>9</sup>/mL tested

| <i>Bacillus</i> spp. tested   | n <sub>2</sub> /n <sub>1</sub> |
|---|--------------------------------|
| <i>B. cereus</i>  | 10/13                          |
| <i>B. subtilis</i>  | 1/7                            |
| <i>B. licheniformis</i>   | 0/5                            |
| <i>B. thuringiensis</i>   | 0/6                            |
| <i>B. anthracis</i>   | 0/8                            |
| <i>B. cereus</i> spores suspension (>10 <sup>9</sup> /mL) diluted 1:100 | 0/6                            |
|   | 11/45                          |

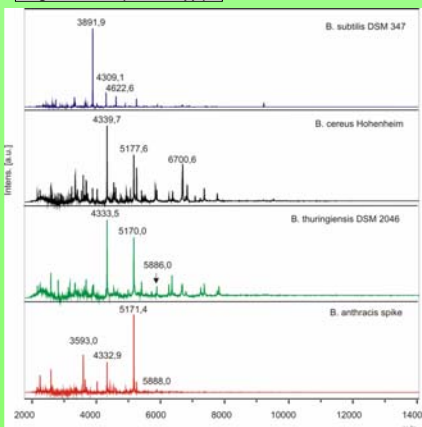
n<sub>2</sub> number of survival after recultivation in enrichment media  
n<sub>1</sub> number of inactivation tests

### Treatment of vegetative cells using TFA (80 %, 30 min)

| Strains tested               | n <sub>2</sub> | n <sub>2</sub> /n <sub>1</sub> |
|------------------------------|----------------|--------------------------------|
| <i>Bacillus</i> spp.         | 8              | 0/1                            |
| <i>B. anthracis</i>          | 5              | 0/7                            |
| <i>Yersinia</i> spp.         | 60             | 0/70                           |
| <i>Y. enterocolitica</i>     | 44             |                                |
| <i>Y. pseudotuberculosis</i> | 14             |                                |
| <i>Y. pestis</i>             | 2              |                                |
| <i>Burkholderia</i> spp.     | 12             | 0/14                           |
|                              |                | 0/102                          |

n<sub>2</sub> number of strains tested  
n<sub>1</sub> number of survival after recultivation in enrichment media  
n<sub>1</sub> number of inactivation tests

### Vegetative cells (*Bacillus* spp.)



### Spores (*Bacillus* spp.)

