MALDI-TOF MS as confirmation tool for pathogens in drinking water

Marsha van der Wiel, Pim Willemse, Gerhard Wubbels
Waterlaboratorium Noord, Glimmen, The Netherlands
Rijksstraatweg 85, 9756 AD. 050 - 402 2121

Introduction

WLN is the center for water quality and water technology. WLN takes care for clean, healthy and tasty drinking water. WLN is progressive in the use of new and faster techniques. One of the newest techniques is the use of the Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) biotyper. WLN is the first water laboratory in the Netherlands that is accredited for the routine use of the MALDI-TOF biotyper. With this new technique WLN can identify indicators and pathogens such as *Escherichia coli*, *Enterococcus* and *Legionella* within a few minutes directly from their selective media.

Validation

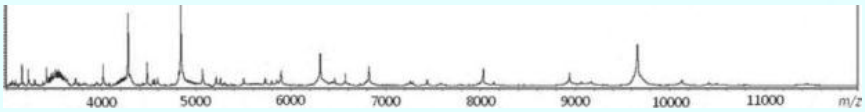
In this table, you can see the conformity between NEN- and MALDI-TOF MS confirmation. The validation is performed according NEN-EN-ISO 16140.

Confirmation	Relative accuracy	Relative specificity	Relative sensitivity
Escherichia coli	100%	100%	100%
Coliforms	100%	100%	100%
Enterococcen	99%	100%	98%
Legionella sp.	93%	95%	92%
Legionella	100%	100%	100%

Legionella is divided in two stages, the genes Legionella scores 100% similarity. Legionella species has a lower score because 4 Legionella species are not present in the Bruker database.

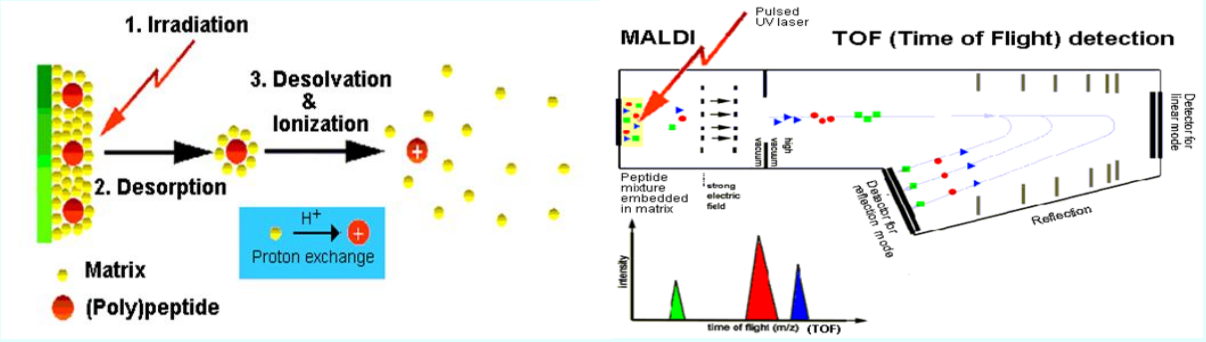
Discussion / Conclusion

Certain peaks of the microorganism 'fingerprint' are conserved, the so called abundant proteins. These proteins are always there and make it possible to characterize microorganisms. Some microorganisms are not detected (Legionella species) by the Bruker database, because Bruker did not introduce these microorganisms. Therefore the Dutch water laboratories will generate a collective 'water database'



Methods

MALDI-TOF MS is based on the chemotaxonomy of microorganisms. A single colony of a target organism is put directly on a 96 target plate. After deposition the spots were overlaid with 1 µl matrix solution (2.5mg α-Cyano-4-hydroxycinnamic solved in 50% acetonitrile, 2.5% trifluoro acetic acid, 47.5% ultra-pure water). The matrix opens the cell wall. A laser irradiate the matrix sample (1), to divide it in little portions of peptides (2). The matrix evaporate and positive charged peptides become free (3). In the strong electric field the positive charged peptides are lined up. So these peptides have the same starting point, before they accelerate in the flight tube to get to their specific time-of-flight corresponding with their specific mass.



Spectra are generated with the MALDI-TOF MS biotyper from Brüker Daltonik GmbH and compared with approximately 4000 spectra in the Brüker Daltonik GmbH database. In a log score 1 to 3 the MALDI-TOF biotyper defind the similarity of the known and unknown spectra . WLN has tested 67 reference strains and 316 samples, grown on their specific and general media. These microorganisms were confirmed with classic techniques and the MALDI-TOF MS biotyper.