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MALDI-TOF MS goes mosquitoes

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MALDI-TOF MS is a versatile technology with many applications. In recent years it has revolutionized microbial identification in the healthcare and industrial sector. Yet, amidst its successes, it's easy for people to overlook that, with the right instrument and supporting software for creating customized reference libraries, the identification capabilities of this technology extend far beyond microbes. Here we would like to highlight the contribution of the MALDI Biotyper in research projects dedicated to reducing the impact of vector-borne diseases.

Mosquitoes, comprising approximately 3,500 recognized species, rank among the most significant invertebrates globally. Their itchy, irritating bites and ubiquitous presence can ruin idyllic settings like candlelit beach dinners or tranquil canoe camping trips by the lake. Depending on the region they can be much more than a nuisance as they also stand as formidable vectors for disease transmission, surpassing all other creatures in this regard. They represent a significant threat to both animal and human health, as well as to economic development.

Mosquitoes possess the ability to detect carbon dioxide emitted by animals. A recent study highlighted the specific recognition mechanism employed by mosquitoes, with

female *Aedes aegypti* mosquitoes displaying a strong preference for human odor over that of other animals.¹

Recent studies have highlighted MALDI-TOF as a rapid and accurate method for identifying invertebrates, along with potentially associated microorganisms. In comparison to sequencing, MALDI-TOF MS emerges as an accessible, cost-effective, and time-saving technique in entomology.

In a recent investigation conducted in Vietnam, where the prevalence of diseases transmitted by mosquitoes is considerable, the MALDI Biotyper was employed to identify these vectors.² This study highlighted how MALDI-TOF MS can prevent misidentification of mosquitoes, compared to morphology-based identifications. The technology surpasses the constraints of traditional morphological and molecular identification methods by eliminating the need for specialized entomological expertise, facilitating training for technical personnel, and streamlining time and cost requirements.

Bruker is supporting customers to generate in-house non-microbiological databases and to enrich these on a continuous level. For good coverage of the local mosquito diversity the customers first need to create reference

spectra for each abundant species, so that later unknown samples can be identified with a high success rate.

MALDI-TOF MS emerges as a novel entomological surveillance tool poised to significantly contribute to the management of mosquito-borne diseases.

In a recent investigation, the MALDI Biotyper was employed to identify mosquito species collected in Chad, a landlocked country in Central Africa. These mosquitoes had been preserved with silica gel for a duration of two months. Through MALDI-TOF MS analysis, researchers not only identified the mosquito species but also determined their blood meals. The analysis revealed that mosquitoes predominantly fed on humans, birds, and cows. The study highlighted MALDI-TOF MS as a promising tool for identifying mosquitoes stored in silica gel and moderately discerning the origin of their blood meals.³

A collaborative effort between France and Mali has enhanced the identification of the origin of blood meals. MALDI-TOF MS has emerged as a rapid, reliable, and cost-effective method for determining the blood meals of *Anopheles gambiae* Giles, *Anopheles coluzzii*, and *Aedes albopictus*. These mosquitoes were either artificially fed with different host bloods sequentially or engorged with mixed bloods from various vertebrate hosts, including humans, sheep, and dogs. Following the expansion of the customized database with new entries, correct identification was achieved in all tested samples.⁴

Another study from India highlighted the flexibility of sample matrices, where legs were substituted with alternative sample matrices. In Chandigarh, India, researchers explored the preparation of cephalothoraxes for mosquito identification.⁵ They collected four genera of medically important mosquitoes from North India and utilized them to create MALDI-TOF MS protein profiles. The spectra generated from nine mosquito species not only exhibited visually distinct characteristics but also featured specific signature peaks.

The MALDI Biotyper proved to be a rapid, accurate, and cost-effective tool for identifying several mosquito species, including *Ae. aegypti*, *Ae. albopictus*, *An. Stephensi*,

An. annularis, *An. culicifacies*, *Ar. subalbatus*, and *Cx. quinquefasciatus*, *Cx. vishnui*, *Cx. tritaenorrhynchus*. With its ease-of-use, its relatively quick turnaround time and minimal running costs, this method demonstrated good efficiency for routine use.

Anophelines are recognized vectors of malaria, *aedine* species transmit dengue and chikungunya, while *culicines* spread filariasis, Japanese encephalitis, and West Nile fever. Given the significant public health concerns associated with mosquito-transmitted diseases worldwide, the rapid and accurate identification of these mosquito species is crucial for implementing effective vector control measures.

The German MALDI platform, MALDI-UP, offers sample preparation protocols for MALDI-TOF MS for insects, known as Ethanol/Formic Acid Extraction - Modified for Insects (EtOH-FA-I), which are freely available for download.⁶ A variety of reference spectra (MSP) and single spectra prepared using this protocol can be accessed on the MALDI-UP homepage (<https://maldi-up@ua-bw.de>). This resource isn't limited to mosquitoes but encompasses a wide array of insect entries.

The MALDI Biotyper streamlines the challenges posed by morphological variations and the complexities associated with DNA-based identification tools.

References

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- ⁶ The MALDI-UP homepage is generated by the State Institute for Chemical and Veterinary Analysis of Food (CVUA) Stuttgart. MALDI-UP Status of 28 November 2023: It includes total 4798 reference entries and 9999 total validation spectra. <https://maldi-up.ua-bw.de/catalogue.asp>.

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