



STSM GRANTEE: Cimpan Andrei

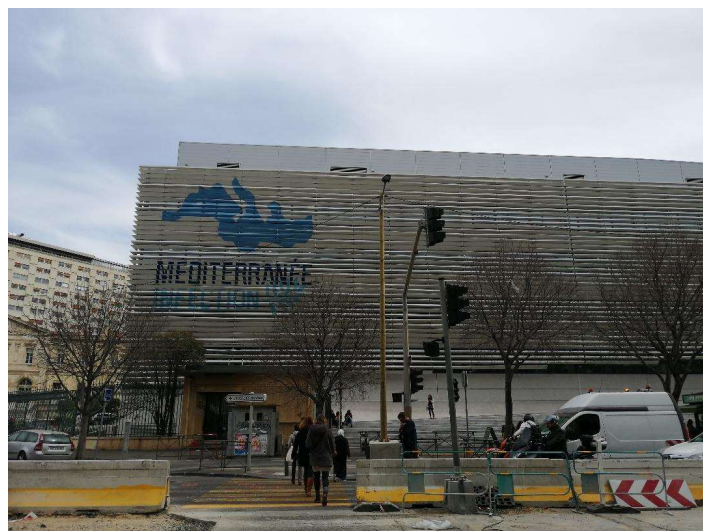
HOST INSTITUTION: Research unit for Vectors - Tropical and Mediterranean Infections (**VITROME**) - Aix Marseille University, France

HOST: Professor Philippe Parola

STSM: COST Action: CA17108, WG 1

DURATION: 15th of March – 12th of April 2019

Identification of mosquitoes collected from Greece by MALDI-TOF MS



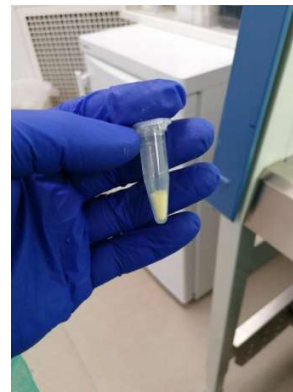
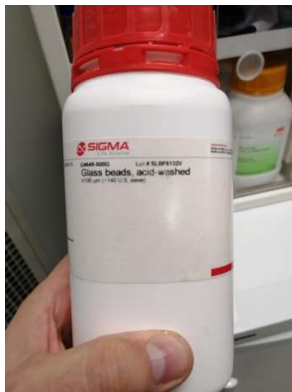
1 Training: Identification of arthropods by MALDI-TOF MS(Matrix-Assisted Laser Desorption Ionization Time of Flight)

The grantee has learned to dissect ticks, lice, mosquitoes and sandflies, in preparation for identification with MALDI-TOF. The grantee also learned the procedures of sample preparation specific for each arthropod species and the preparation of the matrix used in the MS analysis.

	Body part used for MS analysis
Ticks	legs
Mosquitoes	legs
Lice	head and torax
sandflies	torax wings and legs



Automatic homogenisation of the samples using acid washed glass beads and TissueLyser II(Qiagen)



Preparation of the matrix composed of saturated α -cyano-4-hydroxycinnamic acid, 50% acetonitrile and 2.5% trifluoroacetic acid

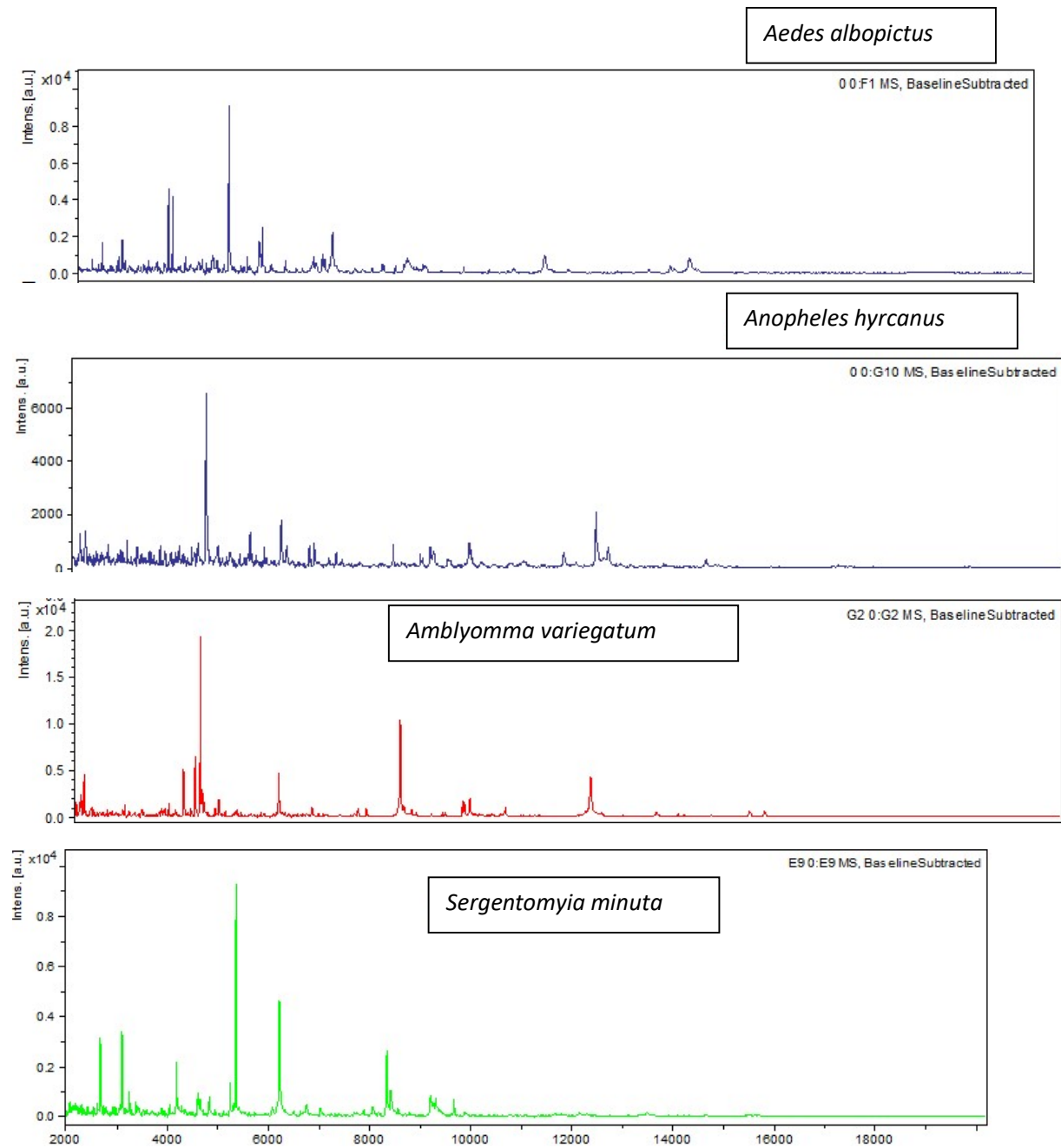


To avoid the destruction of field samples, during the training period the grantee analysed only mosquitoes(*Anopheles coluzzii* and *Aedes albopictus*)[1,2], lice(*Pediculus humanus corporalis*) and ticks(*Rhipicephalus sanguineus* and *Amblyomma variegatum*)[3] from the laboratory colonies.

Only after good quality spectra were obtained using the arthropods from the laboratory colonies, the trainee started to analyse samples from the field. Because field samples were stored in alcohol, the alcohol was removed and samples were first incubated at 37°C over night[4].

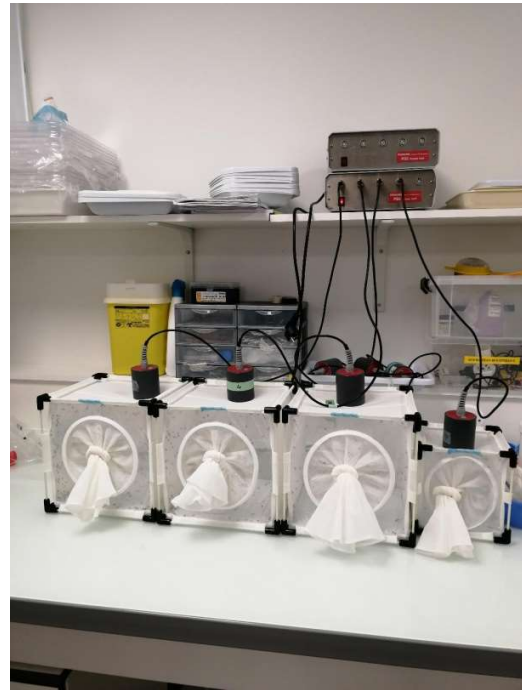
Mandatory trainings on molecular biology, working in the insectarium and the general use of MALDI-TOF were also provided.

Samples of the spectras obtained during the initial training. The quality of these spectra varies because they were obtained during the earlier part of my training:





Aedes albopictus colonies(incubator)



Aedes albopictus colonies(blood feeding)

Colony mosquitoes used for the training

2 Identification of mosquitoes from Greece

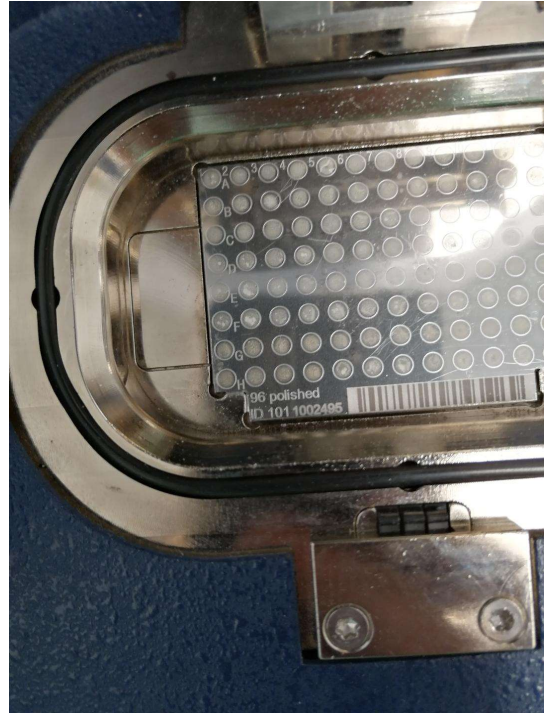
Before processing samples for MALDI-TOF, morphological identification of field mosquitoes was done using MosKeyTool identification key provided by MediLabSecure.

Good spectra were obtained from field samples stored in alcohol in spite of previous reports showing that storage in alcohol can affect the quality and reproducibility of the spectra[4].

Species that are not present in the database or are present but have been manually homogenised or not stored in alcohol will be sequenced and the MS spectra will be added to the database.



**Mass Spectrometer device used for the study
Microflex LT MALDI-TOF (Bruker Daltonics,
Germany)**



**Samples and matrix were loaded on the target
plate. The plate is loaded in the machine and ready
for analysis.**

3 Further collaboration

Both parties are committed to complete current collaborative projects and have already discussed to continue working together using the Bruker Microflex MALDI-TOF present at the University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania, where the trainee is a PhD student.

References

1. Diarra AZ, Laroche M, Berger F, Parola P. Use of MALDI-TOF MS for the identification of Chad mosquitoes and the origin of their blood meal. *Am J Trop Med Hyg.* 2019;
2. Tandina F, Niaré S, Laroche M, Koné AK, Diarra AZ, Ongoiba A, et al. Using MALDI-TOF MS to identify mosquitoes collected in Mali and their blood meals. *Parasitology.* 2018;
3. Boucheikhchoukh M, Laroche M, Aouadi A, Dib L, Benakhla A, Raoult D, et al. MALDI-TOF MS identification of ticks of domestic and wild animals in Algeria and molecular detection of associated microorganisms. *Comp Immunol Microbiol Infect Dis.* 2018;
4. Diarra AZ, Almeras L, Laroche M, Berenger JM, Koné AK, Bocoum Z, et al. Molecular and MALDI-TOF identification of ticks and tick-associated bacteria in Mali. *PLoS Negl Trop Dis.* 2017;