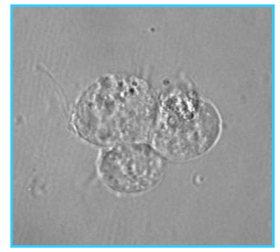
MALDI-TOF mass spectrometry for the identification of *Trichomonas vaginalis*: preliminary study.

Adriana Calderaro, Maddalena Piergianni, Mirko Buttrini, Sara Montecchini, Giovanna Piccolo, Sabina Rossi, Flora De Conto, Maria Cristina Arcangeletti, Maria Cristina Medici, Carlo Chezzi.

Unit of Microbiology and Virology, Department of Clinical and Experimental Medicine, University of Parma, Italy.

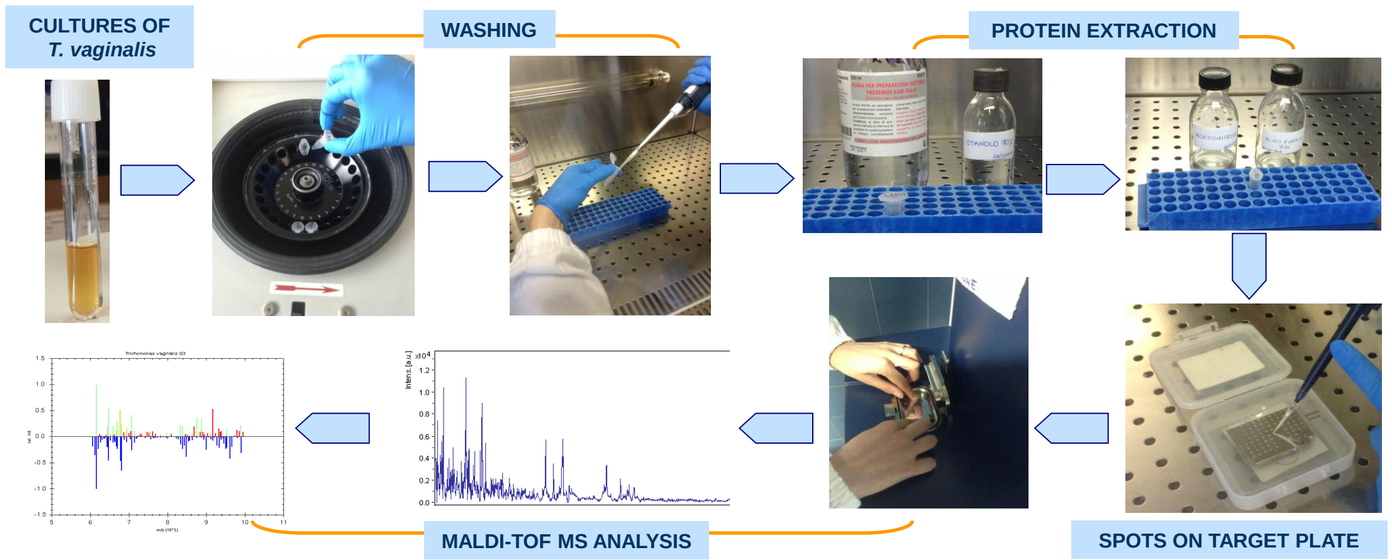
25th ECCMID COPENHAGEN, DENMARK 25-28 APRIL 2015

Introduction and purpose. *Trichomonas vaginalis* is a flagellated protozoa causing trichomoniasis, a common but overlooked sexually transmitted human infection, with around 170 million cases occurring annually worldwide. In diagnostic practice, the gold standard for the identification of *T. vaginalis* is the visualization of motile parasites by microscopy after culture of the clinical sample on specific media. Recently, immunochromatographic assays for the detection of specific antigens, as well as PCR assays for the simultaneous detection of the DNA of *T. vaginalis* and other sexually-transmitted bacteria and viruses, are also available. The well-known genome sequence of *T. vaginalis* has made possible the application of proteomic methods to the studies focused on specific proteins of this parasite. However, few data are available about the overall proteomic expression profiling of *T. vaginalis*. The aim of this study was to obtain a proteic profile to identify *T. vaginalis* field isolates by Matrix-assisted laser desorption/ionization Time-of-Flight mass spectrometry (MALDI-TOF MS).



(Modified from: Calderaro A, 2012)

Methods. In this preliminary study, MALDI-TOF MS was applied on the reference strain *T. vaginalis* G3 cultivated in TYM2 medium and on 4 field strains isolated in our laboratory from clinical samples by using "*Trichomonas* medium N.2" (Oxoid) and identified by microscopic examination. Aliquots (1.5 ml) of cultures of all these strains were submitted to formic acid/acetonitrile protein extraction. The spectra obtained with Microflex LT mass spectrometer (Bruker Daltonics, Germany) were analysed by FlexAnalysis software. The spectrum obtained by the reference strain was used to create a Main Spectrum Profile (MSP-Spectrum) to be subsequently supplemented in the Bruker Daltonics database (version 3.1.2.66), not including any protozoa profiles.



Results. The reference strain yielded a proteic profile (Figure 1) that matched none of the existing profiles in the Bruker Daltonics database and no interference with the peaks of the 2 different culture media used in this study was found. This protein profile was found to be reproducible over a second independent experiment analysing at least 10 replicates/time and no differences were observed when this strain was grown in different lots of media. The G3 MSP-Spectrum was supplemented in the Bruker Daltonics database to be used for further blind identification of additional *T. vaginalis* isolates. The 4 field strains yielded an identifiable protein profile with an identification score value >1.7 (Table 1). Some differences among the proteic profiles of these 4 field isolates were observed.

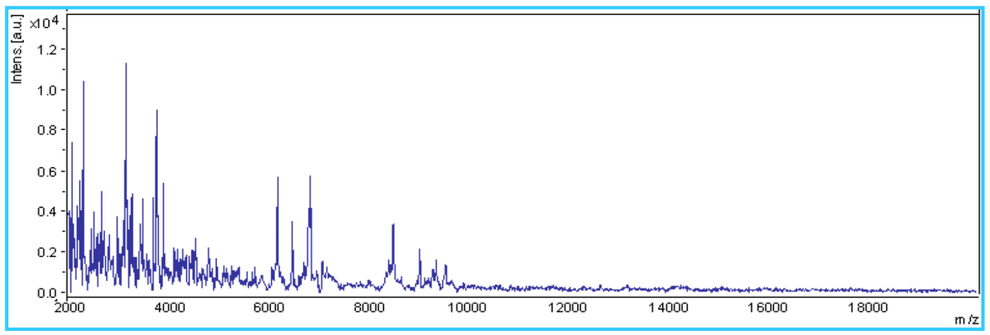


Figure 1. Spectrum of *Trichomonas vaginalis* G3 reference strain.

Table 1. Results of MALDI-TOF MS analysis for the 4 *T. vaginalis* field strains.

FIELD STRAINS	IDENTIFICATION BY CULTURE	IDENTIFICATION BY MALDI-FOF MS	
		Identification	Score
T. vaginalis N. 1	*T. vaginalis*	*T. vaginalis*	1.7<Score<2.0
T. vaginalis N. 2	*T. vaginalis*	*T. vaginalis*	1.7<Score<2.0
T. vaginalis N. 3	*T. vaginalis*	*T. vaginalis*	1.7<Score<2.0
T. vaginalis N. 4	*T. vaginalis*	*T. vaginalis*	1.7<Score<2.0

Conclusions. These results obtained testing 4 *T. vaginalis* field strains isolated from clinical samples are very encouraging because they showed the ability of MALDI-TOF MS to correctly identify this protozoa even if some differences in the proteic profile of the different strains were observed. This preliminary study will be extended by testing the strains isolated in our laboratory during the period 2003-2014 in order to strengthen our results and to subsequently assess the usefulness of this technology in the identification of this protozoan directly on clinical samples.

References. 1) De Jesus JB, Cuervo P, Junqueira M, Britto C, Costa Silva-Filho F, Sabóia-Vahia L, González LJ, Barbosa Domont G. (2007) Application of two-dimensional electrophoresis and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for proteomic analysis of the sexually transmitted parasite *Trichomonas vaginalis*. J. Mass Spectrom.; 42: 1463–1473. 2) Calderaro A, Piccolo G, Montecchini S, Buttrini M, Gorrini C, Rossi S, Arcangeletti MC, De Conto F, Medici MC, Chezzi C. (2013) MALDI-TOF MS analysis of human and animal *Brachyspira* species and benefits of database extension. J. Proteom.; 78: 273-280. 3) Calderaro A, Gorrini C, Piccolo G, Montecchini S, Buttrini M, Rossi S, Piergianni M, Arcangeletti MC, De Conto F, Chezzi C, Medici MC. (2014) Identification of *Borrelia* species after creation of an in-house MALDI-TOF MS database. PLOS One; 9: e88895.