# USE OF MALDI-TOF MASS SPECTROMETRY FOR ANALYSIS OF VIRUS-INFECTED CELLS: A PRELIMINARY REPORT.

**Adriana Calderaro, Maria Cristina Arcangeletti, Federica Motta, Chiara Gorrini, Carlo Chezzi.**

**Department of Pathology and Laboratory Medicine, Section of Microbiology, University of Parma, Viale A. Gramsci 14, Parma, Italy.**

## INTRODUCTION AND PURPOSE

The identification of viral infection traditionally relies on direct methods based on cell culture, antigen or nucleic acid detection which are time consuming and often expensive. This study aimed to demonstrate the ability of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) in detecting specific biomarkers to discriminate between uninfected and virus-infected cells, not yet investigated for diagnostic purposes.

Adenoviruses are common viruses that can cause a broad spectrum of diseases (respiratory illness, fever, diarrhoea, conjunctivitis, cystitis or rash in humans), even if most illnesses are not serious. Because of its fast growth in cell culture producing an evident cytopathic effect, Adenovirus has been used in this study as an experimental model in order to obtain results to be subsequently extended to different viruses of medical interest.

## METHODS

**Cellular culture infection**. Confluent Intestine 407 cells (ATCC CCL-6), grown at 37°C for 48h in a 24-well plate or in 75 $cm^2$ bottle, were inoculated with Adenovirus 18 (ADV18, ATCC VR-19). After a 45-minutes absorption, the viral inoculum was replaced with a maintenance medium (E-MEM medium without serum). Infected cells were incubated at 37°C for 24, 48 and 72 h post infection (p.i.).

**Protein extraction**. At different times post-infection (p.i.), the infected cells and uninfected control culture were washed twice with phosphate buffered saline (PBS), harvested in 300 µl of distilled water and the proteins were extracted by using formic acid and acetonitrile, following the manufacturer's protocol (Bruker Daltonics, Germany). ADV purified particles, obtained from cultures 72 h p.i., were subjected to the same extraction protocol. One microliter of proteic extracts was transferred into the target plate and matrix (satured solution of a cyano-4-hydroxicinnamic acid in 50% acetonitrile) was added, followed by crystallization and air-drying.

**MALDI–TOF analysis**. Samples were analysed on a Microflex LT mass spectrometer (Bruker Daltonics Germany by Becton Dickinson Italia S.P.A.). Spectra, recorded in positive linear mode within a mass range from m/z 2 to 20 Kda, were analysed by Flex Analysis and Maldi Biotyper 3 software (Bruker Daltonics Germany by Becton Dickinson Italia S.p.A).

## RESULTS

Normal and ADV-infected cells were examined by MALDI-TOF at 24, 48 and 72 h p.i.. An aliquot of purified ADV after 72 h p.i. was also analyzed.

The spectra generated from all proteic extracts were analysed in order to investigate the presence of potential biomarkers for ADV-infected cells identification. The spectra obtained from the analysis of uninfected cells were used as the baseline for the detection of any significant protein composition change into the ADV-infected cells, resulting from the inhibitory effect of the viral infection on the cellular protein synthesis or from the synthesis of viral specific proteins.

Figure 1 shows representative spectra of Intestine 407 uninfected and infected cells examined at 24 h, respectively: most of the peaks remained unchanged in mass value and show only little variation of intensity. At this time, it is likely that structural viral proteins have not yet produced.

At 48 and 72 h p.i. (Figure 2) two significant peaks appeared at m/z 2992 and 3169 in the spectra of ADV–infected cells which are completely missing in uninfected cells and overlapping those obtained by the analysis of purified viral particles. In addition some peaks expressed in all uninfected cells spectra (at m/z 2466, 4937, 4962) are not present in the infected cells.

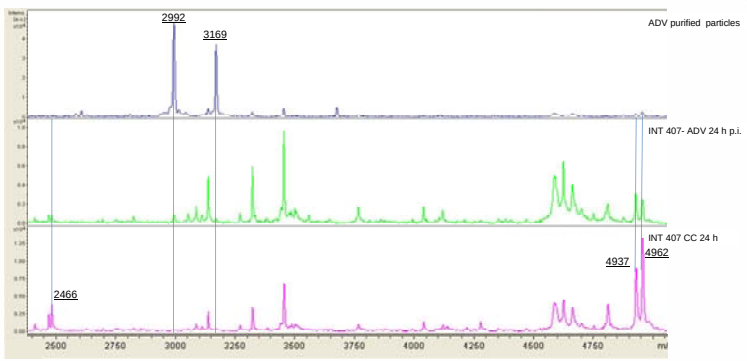


Figure 1. MALDI-TOF spectra in the region m/z 2000 – 5000 of Adenovirus purified particles, intestine 407-infected cells with Adenovirus for 24h, intestine 407 uninfected control cells (CC).

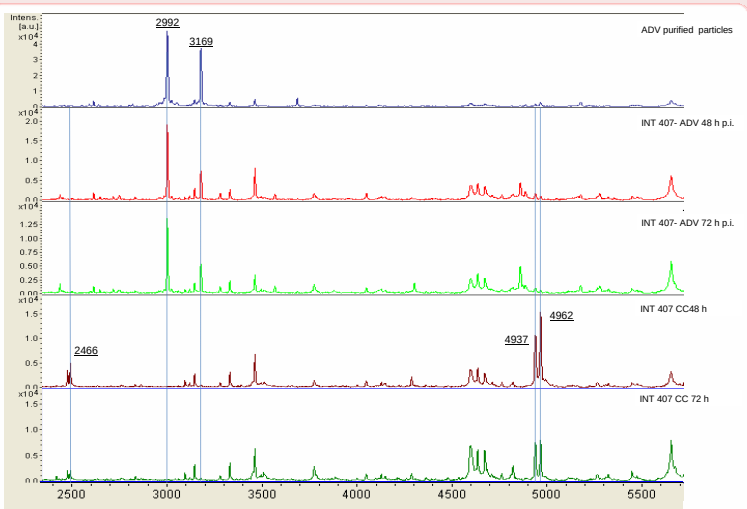


Figure 2. MALDI-TOF spectra in the region m/z 2000 – 5500 of Adenovirus purified particles, Intestine 407-infected cells with Adenovirus for 48 and 72h, Intestine 407 uninfected control cells (CC).

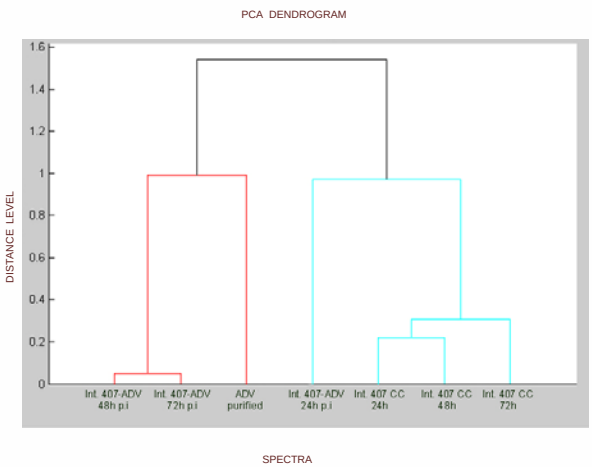


Figure 3 – Dendrogram obtained from the analysis of spectral Principal Components (PCA) of purified ADV particles and of uninfected cells (CC) and ADV – infected Intestine 407 cells at different times p.i.

The analysis of the Principal Components (PCs) of the spectra obtained from the ADV purified particles, uninfected and infected cells at different times p.i. shows the presence of 4 different clusters: the dendrogram in Figure 3 particularly shows a close correlation between the cluster of the spectra obtained from ADV-infected cells at 48 and 72 h p.i. and those from viral purified particles. Similarly, the uninfected cell spectra cluster correlate with those obtained from the ADV infected cells at 24 h.

## CONCLUSIONS

The results obtained in the present study proved the ability of MALDI-TOF analysis to discriminate between uninfected and adenovirus-infected cells. Two unique peaks appeared at m/z 2993 and 3169 in the spectra obtained from the cultures infected with adenovirus at 48h and 72h p.i. and from viral purified particles and it is likely that these proteins are viral biomarkers. On the other hand, some peaks which appeared in all uninfected cells spectra (at m/z 2466, 4937, 4962) are not present in the infected cells. All changes in cellular protein level in infected cells compared to the uninfected cells may be considered as evidence of inhibition of cellular protein synthesis by adenovirus. Although the results obtained in this study are preliminary and should be confirmed using also different virus-cell models, the spectral differences observed between uninfected and virus-infected cells may be a promising basis for the protein spectroscopic detection and identification of infected cells with different viruses in clinical virology.

**References**

Erukhimovitch V., Karpasasa M., Huleihel M. Spectroscopic Detection and Identification of Infected Cells with Herpes Viruses. Biopolymers, 2008, 91(1): 61-67