P–21; **POTENTIAL APPLICATION OF HIGH RESOLUTION MELTING ANALYSIS IN HPV VARIANTS DETERMINATION**

Ivan Sabol, Maja Cretnik, Magdalena Grce
Rudjer Boskovic Institute, Zagreb, Croatia

Background: Nowadays more than 100 HPV types are well characterized. Of about 40 mucosal (genital) types approximately 15 are classified as high-risk (HR), which are the necessary etiological factor of cervical cancer. Accumulating epidemiological data also suggests that viral genome "variants", which diverge by about 2% within a given type and which differ geographically, could contribute to the carcinogenic potential of specific HR HPV types presumably by altering its transforming properties and/or immunogenicity. Most of the studies on HPV-16 variability were concerned with E6 and E7 regions that encode for well known HPV oncogenes. Such studies are very laborious and expensive as several genomic regions of each sample has to be sequenced to detect those few nucleotides that differ and separate the variants.

Objectives: To try to simplify and reduce the costs of variant detection we investigated the prospect of possible application high resolution melt (HRM) analysis in determining HPV-16 variants in E6/E7 junction region.

Materials and Methods: The method consists of adding a double strand specific fluorescent dye to the PCR reaction, post PCR thermal melting of the amplicon and reading of fluorescence emitted by the dye at each temperature point in high resolution. The resulting melting curves are subsequently analyzed by the software provided by manufacturer (Idaho Technology Inc., USA). In theory, HRM analysis enables the distinction of PCR amplicons differing in even a single nucleotide, and as such should be capable of indicating HPV variants. In this feasibility study, we tested the performance of HRM analysis with our regular HPV-16 type specific primers (E6/E7 region) on 81 HPV-16 positive cervical samples from Croatian women.

Results: Eight amplicons exhibiting melting curves that were deviating slightly or not at all (dominant pattern) and only one amplicon deviating greatly from the average melting curves were sequenced. The sequence of the dominant pattern samples corresponded the European HPV-16 variant, while the clearly different one was related with the African HPV-16 variants. Thus, only those samples whose amplicon melting curves differ significantly from the dominant curves (in our case European variant) should be sequenced to identify the HPV variant.

Conclusions: This approach seems to be very informative, saves time and significant cost of sequencing. The method will be optimized using amplicons from more variable regions of E6/E7 in respect to different HPV-16 known major variants.