
NEW HIGH-THROUGHPUT METHOD FOR ONCOGENIC HUMAN PAPILLOMA VIRUSES GENOTYPING BY MULTIPLEX FLUORESCENT PCR (F-HVP TYPING) IN 4051 CERVICAL SAMPLES

Paz Cañadas¹, Vincenzo Cirigliano¹, Elena Ordoñez¹, Maijo Ejarque¹, Gianfranco Voglino²

¹*General Lab, Barcelona, Spain*

²*Promea Lab, Turin, Italy*

Background: Human Papillomavirus (HPV) infection has been well established as etiological agent of cervical cancers. Introduction of HPV genotyping either in cervical screening programs or for monitoring the effectiveness of HPV vaccination, requires access to fast, simple and high-throughput technology.

Objectives: Our objective was to evaluate a new multiplex fluorescent PCR method for HPV detection and typing assessing high risk HPV frequency and relationship with cytological and histological data.

Materials and Methods: Cervical specimens were collected from 4,051 Italian women (15–65 years aged) with abnormal cytological smears and were tested for the presence of HPV using the f-HPV typing Kit, a recently available commercial assay (Molgentix Barcelona). Extracted DNA was amplified with 15 sets of fluorescent labelled primers recognising HPV type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 6 and 11. Labelling of primers with 6-FAM, NED, VIC and PET allowed high sensitivity as well as amplification of similar size products in the same PCR reaction. After electrophoresis on ABI 3130 DNA sequencer, PCR products were automatically recognised by size and colours. Cytological and histological analysis was performed.

Results: High risk HPV types were detected in 80% (1,145/4,091) and low risk HPV in 20% (284/4,091) of the samples. High risk HPV were detected in 42% (43/102) of ASCUS-AGUS, 50% (234/466) of CIN1, 70% (88/126) of CIN2, 97% (35/36) CIN3, 98% (118/120) of squamous cervical cancer and finally in 100% (25/25) of adenocarcinomas. HPV-16 was the most prevalent type in LSILs (40%), in HSILs (72%), and in carcinomas (62%) followed by HPV-31.

Conclusions: The f-HPV typing™ kit is an accurate technique for quick and sensitive detection of 15 HPV types, it also allows distinguishing single and multiple infections in several cases. The high throughput of the assay allows assessing different HPV types frequency in a population and may be of help in determining possible changes that could derive as consequence of vaccination policies.