CLINICAL VALIDATION OF THE REALQUALITY RQ-HPV HR MULTIPLEX ASSAY BASED ON MEIJER'S GUIDELINES

1)Dalla Pozza A.. 2)Tiozzo S.. 3)Bortolozzo K.. 4)Mason S.. 5)Paladin D.. 6)Giusti P..

Università degli Studi di Padova

The key issue for high risk Human Papillomavirus (hr-HPV) DNA testing in cervical cancer screening is to detect hr-HPV infections that are associated with or develop cancers with a CIN (cervical intraepithelial neoplasia) index ≥ 2 and to differentiate them from transient hr-HPV infections. A clinical method that aims to detect hr-HPV infections should have a balance between clinical sensitivity and specificity to identify only CIN ≥ 2 samples (Meijer et al., 2008).

Meijer's guidelines give defined values about the specificity, the sensitivity and the intra-laboratories reproducibility, necessary for the validation of a new assay.

The REALQUALITY RQ-HPV HR Multiplex assay is used for detection of the DNA of 14 high-risk genotypes of Human Papillomavirus and genotyping of HPV 16 and HPV 18. This in vitro diagnostic test for detection of Human Papillomavirus is an auxiliary device for diagnosis and monitoring of HPV infections. The test is based on Real-Time PCR on DNA extracted from human clinical samples. For the evaluation of the clinical sensitivity and specificity of the REALQUALITY RQ-HPV HR Multiplex device the HC2 test (hc2 High-Risk HPV DNA Test, QIAGEN) was used as a reference method (FDA-approved assay). 956 samples were selected for the study: 73 of them were samples with an identified CIN index ≥ 2 whereas the remaining 883 samples have been used as control, given that were samples with a CIN index < 2. HC2 and REALQUALITY RQ-HPV HR Multiplex tests were simultaneously performed on these samples.

The REALQUALITY RQ-HPV HR Multiplex assay showed 100% sensitivity for CIN2+ relative to that of HC2: 71/73 samples were hr-HPV positive for both the assays. Two samples were HPV negative and this result is not concordant with the histological data. The clinical specificity of REALQUALITY RQ-HPV HR Multiplex relative to HC2 and tested with the samples < CIN2 was 99.74%. In particular, 15 positive samples with HC2 test resulted as negative with REALQUALITY RQ-HPV HR Multiplex and 17 negative samples with HC2 test resulted as positive with REALQUALITY RQ-HPV HR Multiplex (data in press). Since the device for screening is not able to genotype all the hr-HPV, the aim of this study is the analysis of the genotypes present in the HPV positive samples. These data are important in order to understand the discrepant results obtained and also for the analysis of hr-HPV distribution. In this study all the HPV positive samples are going to be genotyped on L1 region using an assay based on Reverse Line Blot (AMPLIQUALITY HPV TYPE EXPRESS, AB ANALITICA).

Meijer et al., (2008). Guidelines for human papillomavirus DNA test requirements for primary cervical cancer screening in women 30 years and older. 124, 516–520