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In Situ Detection of HPVs on Clinical Specimens Using Fully Automated Xmatrx $^{f R}$ System

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Introduction

Human papillomavirus (HPVs) induce a variety of proliferative lesions, leading to the onset of squamous cell carcinoma (SCC) in various tissues. The 14 genotypes of high-risk HPVs, including 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68, have been strongly associated with risk of development of cervical malignancy. Hence, high-risk HPV screening is very important for setting clinical intervention strategies to prevent progression of cervical carcinoma.

BioGenex, has developed 14 high-risk HPV cocktail detection system, which can be used on fully automated Xmatrx[®] platform. A highly specific and sensitive assay based on Chromogenic In situ Hybridization (CISH) technique which also preserves the tissue morphology.

Methods

Cohort of 278 histopathologically confirmed invasive SCCs were used for the study.

Additional investigation was carried out on normal, low & high grade squamous intraepithelial lesions (LSIL and HSIL) and cervical scrapes. The sensitivity of the assay was ascertained by performing the staining using cervical cancer cell lines containing a known copy number of HPV 16 (SiHa 2-5 copies) and HPV 18 (HeLa 10–50 copies).

HPV 14 high risk cocktail probe (BioGenex PR251) was hybridized to the exposed target DNA sequences in infected cells, which was detected using In situ Hybridization Detection Kit (BioGenex # DF330-YADE). The kit comprised of sequential addition of anti-fluorescein antibody followed by Poly-HRP labeled secondary antibody which allows for signal amplifications and final color development with DAB chromogen.

The assay was performed on BioGenex Xmatrx[®]- a fully automated walk-away staining system.



In Situ Detection of HPVs on Clinical Specimens Using **Fully Automated Xmatrx[®] System**

HPV CISH Assay using Xmatrx®

FFPE Tissue sections/Cervical scrapes/cell lines Nucleic Acid Retrieval (Performs Dewax and Pretreatment) HPV probe (14 high risk cocktail probe) Hybridization Stringency washes Anti Fluorescein antibody Poly-HRP labeled secondary antibody DAB Chromogen Counterstain Final Cover slipping and mounting

Results

HPV 14 high-risk probe and ISH detection system proved to be a powerful technique for localizing specific nucleic acid sequences within cells of tissues and cytological specimens.

HPV infection was detected in 272 of 278 cervical carcinomas, 2 of 2 LSIL, 2 of 2 HSIL, and 17 of 64 cervical scrapes.

The sensitivity of BioGenex's HPV 14 HR probe is found to detect HPV at both very low copies (2-5 copies) in SiHa cells and low copies (10-50 copies) in HeLa cells.













SiHa Cell Line

Conclusion

- and researchers.



Staining pattern of HPV 14 HR genotypes using CISH assay

Carcinoma of Cervix





Cervical scrapes

Punctuate signal of HPV 16 and 18 using CISH assay in Cell lines



HeLa Cell Line

• Our results confirm that HPV CISH detection system using a fully automated instrument is highly sensitive and specific and can be used to visualize HPV infected cells in FFPE Tissues and cytological specimens.

• HPV 14 high-risk detection system platform is the first of its kind catering to the needs of both clinicians