Detection of HPV16/18 in Cervical Carcinoma Using Fully Automated in situ PCR System

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Detection of HPV16/18 in Cervical Carcinoma Using Fully Automated *in situ* PCR System

Abstract # 1937
Poster # 259

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Introduction

Molecular detection of Human Papilloma Virus (HPV) DNA is currently the gold standard for detection of HPV. Various methods that are in use include, dot blotting, southern blotting, chromogenic ISH (CISH), hybrid capture and PCR. Unlike conventional methods like PCR, *in situ* PCR (ISP) can help in detection of low copy nucleic acid targets (DNA/ mRNA) while preserving the histological context.

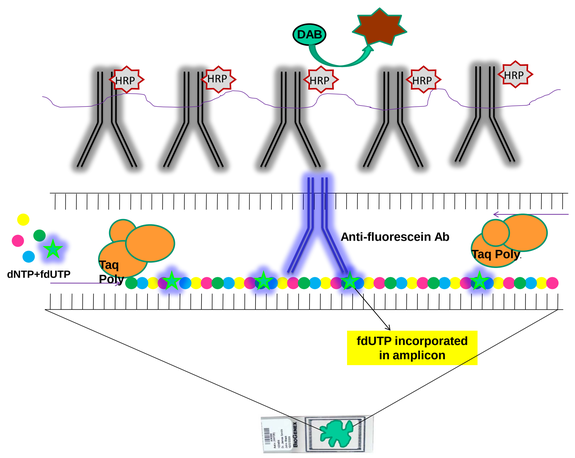
In this study, we have reported the use of fully automated detection of HPV16 in cervical carcinoma tissue using ISP and established concordance between ISP and CISH

Methods

Cohort of 20 cervical carcinoma samples is used for the study. SiHa cell lines (1-2 copies of HPV16) and HeLa cell lines have served as experimental control

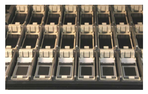
In order to verify the specificity, tissues from normal cervix, breast carcinoma and colon carcinoma were also tested.

PCR primers were designed to amplify the E6 region of HPV16 and HPV18. Fluorescein labeled dUTP's are incorporated during PCR amplification and subsequently, amplicons were detected with sequential addition of anti-fluorescein antibody, and a poly-HRP labeled secondary antibody followed by final color development with DAB chromogen (BioGenex Kit # DF300).



Methods

Tissue Sections
↓
Ez-Dewax
↓
Nucleic acid retrieval
↓
in situ PCR master mix
↓
20 cycle PCR
Fluorescein-dUTP incorporated
↓
Anti-fluorescein Ab
poly-HRP Detection System
DAB chromogen



Xmatrx Infinity

Results

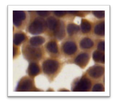
All the 20 cervical carcinoma samples in this cohort detected positive for HPV16 by ISP and 15/20 are found to be positive by CISH.

The results showed 75% of concordance between HPV16 ISP and HPV16 CISH using automated detection system.

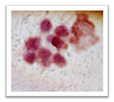
The sensitivity of this detection is found to be as low as 1-2 copies of HPV16 using SiHa cell lines.

All the 20 cervical carcinoma samples were also found positive or

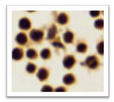
In addition, HPV18 were detected in HeLa cells with more copy numbers than those of HPV16 in SiHa cells.



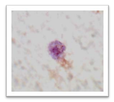
***in situ* PCR**
(HPV16) 20 cycles
SiHa cell line



***in situ* Hybridization**
(HPV16)
SiHa cell line



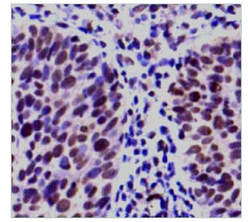
***in situ* PCR**
(HPV18)20 cycles
HeLa cell line



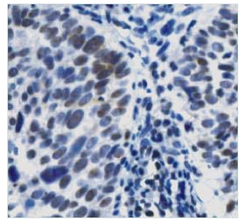
***in situ* Hybridization**
(HPV18)
HeLa cell line

Results

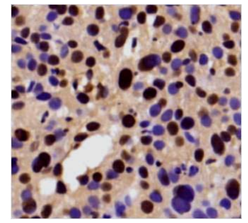
A) ***in situ* PCR** (HPV16)



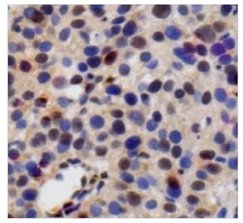
B) ***in situ* Hybridization** (HPV16)



C) ***in situ* PCR** (HPV18)



D) ***in situ* Hybridization** (HPV18)



A) Cervical carcinoma showed positive signals after 20 cycles *in situ* PCR for HPV-16.
B) HPV16 detected by CISH.
C) Cervical carcinoma showed positive signals after 20 cycles *in situ* PCR for HPV-18.
D) HPV18 detected by CISH.

Conclusions

Fully automated ISP detection is observed to be 25% more sensitive when compared to CISH with an ability to detect even 1-2 copies of HPV16 using SiHa cell lines.

Fully automated ISP offers rapid and sensitive method for histological localization of HPV16/18.

Further, this is useful for the early screening of HPV infection in cervical scrapings and cervical intraepithelial neoplasia, where early detection of very small quantities of HPV is critical for prediction of clinical outcome.