Detection of Human Papillomavirus (HPV) mRNA in FFPE Samples Using AMPIVIEW™ RNA Probes, Powered by Enzo's LoopRNA ISH™ Technology

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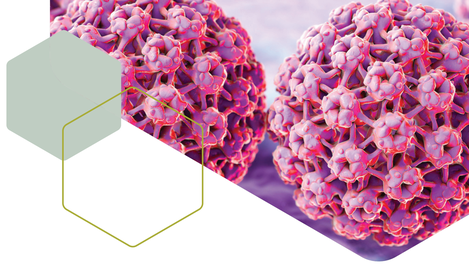


ABSTRACT

Human papillomavirus (HPV) infection is associated with a variety of clinical conditions that range from innocuous lesions to cancer. *in situ* hybridization (ISH) is a powerful tool used in clinical and research labs for the detection of HPV infection in formalin-fixed paraffin-embedded (FFPE) tissue samples and cells. HPV detection varies among methods due to HPV copy numbers in tissues or insufficient specificity and sensitivity of the assays. This study will introduce the new AMPIVIEW™ RNA probes used to detect high-risk and low-risk HPV infections. AMPIVIEW™ HPV RNA probes were uniquely designed with the precision of targeted, sequence-specific RNA probes powered by Enzo's LoopRNA ISH™ technology to deliver superior sensitivity. Results with the AMPIVIEW™ RNA probes show that ISH sensitivity matches to PCR sensitivity when tested in high-grade squamous intraepithelial lesion (HSIL) samples. While PCR assays require the homogenization of the samples, ISH results can be observed under a light microscope without disrupting the morphology of the sample. Finally, scientists can visualize the expression and spatial localization of their target genes with ease.

INTRODUCTION

Papillomaviruses are small, non-enveloped icosahedral viruses, possessing a circular double-stranded DNA (dsDNA) genome of about 8 kb in length[1]. More than 200 types of HPV have been identified. HPVs can be grouped as high-risk (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82) and low-risk (HPV types 6, 11, 42, 43 and 44)[2]. The two most common HPV high-risk genotypes are HPV 16 and HPV 18, which cause approximately 70% of all cervical cancers[3].

Persistent infections with high-risk HPV strains can lead to precancerous lesions which may progress to cervical cancer. HPV-high-risk are responsible for more than 99% of cervical cancer cases, of which 55% are HPV type 16 and 15% are HPV type 18. HPV 16 and HPV 18 genotypes are responsible for about 50% of cervical intraepithelial neoplasia (CIN), about 70% of cervical cancers and a growing number of oropharyngeal cancers[3]. Furthermore, HPV high-risk infection is associated with cancers at a variety of other anogenital sites: around 50% of penile, 25% of vulvar, 80% vaginal, and close to 90% of anal cancers[4].

Advances in nucleic acid sequencing and RT-PCR enabled the research community to investigate in great detail, the mechanisms and regulations of HPV gene expression and replication during the early and late phases of viral infection. Sequencing and RT-PCR are very sensitive tools and can be used to detect a very small amount of HPV nucleic acid molecules, but they lack cell morphology and spatial localization of its targets. Those are important parameters for the evaluation of cervical specimens for HPV-induced cancers.

in situ hybridization (ISH) is a widely used laboratory technique for the detection of nucleic acids molecules in cells and tissue specimens. Unlike sequencing and RT-PCR techniques, which require sample homogenization, ISH techniques preserves the morphology of the sample and can provide target detection at the subcellular level.

One of the main challenges for ISH applications is sensitivity, especially for those involving the detection of single-copy targets. In clinical settings, insufficient sensitivity can lead to misdiagnosis. To overcome this limitation, Enzo developed AMPIVIEW™ RNA probes, powered by Enzo's LoopRNA ISH™ technology to deliver high sensitivity to visualize the spatial biology of nucleic acids in the sample.

REFERENCES

1. Doorbar J, et al. Human papillomavirus molecular biology and disease association. *Rev Med Virol* 2015:25 Suppl 1:2-23
2. Munoz N, et al. International agency for research on cancer multicenter cervical cancer study G. epidemiologic classification of human paillomavirus types associated with cervical cancer. *N Engl J Med* 2003; 348:518-527
3. Graham, SV. The human papillomavirus replication cycle, and its links to cancer progression: a comprehensive review. *Clin Sci* 2017; 131:2201-2221.
4. de Martel C, et al. Worldwide burden of cancer attributable to HPV by site, country and HPV type. *Int J Cancer* 2017; 141:664-670
5. La Rocca G, et al. Recent improvements in *in situ* hybridization for the detection of HPV infetions in clinical samples. *World Cancer Research Journal*, WCRJ 2020; 7e1542

METHODOLOGY

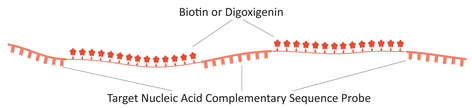
AMPIVIEW™ RNA PROBES
POWERED BY ENZO'S LOOPRNA ISH™ TECHNOLOGY

Enzo's LoopRNA Design

Nucleic Acid Target

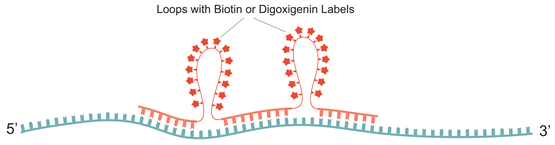
HPV low-risk (HPV 6, HPV 11) and high-risk (HPV 16, HPV 18, HPV 31, HPV 33, HPV 51) genotypes are chosen as targets for the probes.

AMPIVIEW™ RNA Probes



Analyze sequence of the target nucleic acid and design the probes. Probes are produced with reporter enzymes conjugated between complementary bases. Sense RNA probes can detect DNA while anti-sense RNA probes can detect DNA and RNA in the cells.

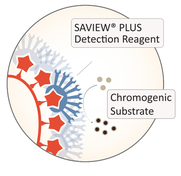
Hybridization



During hybridization, AMPIVIEW™ RNA probes form loops exposing biotin or digoxigenin labels. Labels can be detected with antibodies against biotin or digoxigenin and amplified with immunohistochemistry detection reagents.

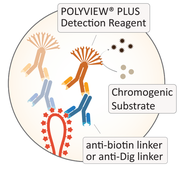
Detection

Biotin-conjugated Probes



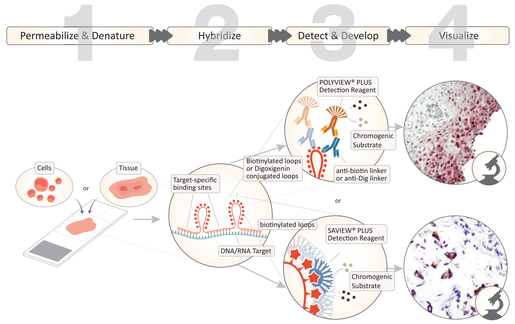
SAVIEW® PLUS AP or HRP reagent is a strepavidin-based nanopolymer detection solution to ensure consistent and reproducible detection of biotinylated probes on tissues and cells. SAVIEW® one-step detection method for biotinylated AMPIVIEW™ RNA probes enables faster staining procedures with significantly lower background.

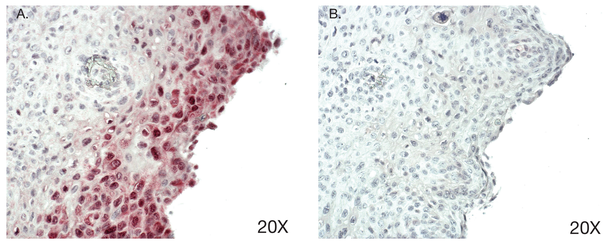
Biotin- or Digoxigenin-conjugated loops



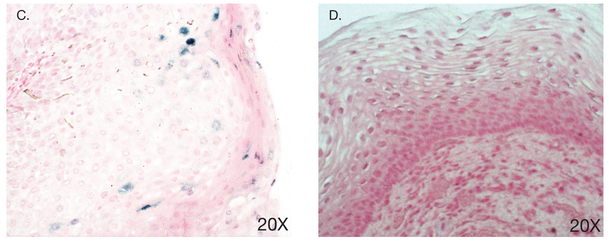
POLYVIEW® PLUS AP or HRP reagent, a biotin-free nanopolymer solution, combined with anti-biotin or DIGX® anti-digoxigenin linkers, produces consistent and reproducible detection of AMPIVIEW™ RNA probes. POLYVIEW® PLUS high intensity color development with HIGHDEF® chromogens, delivers sharp and crisp stainings.

AMPIVIEW™ ISH Workflow

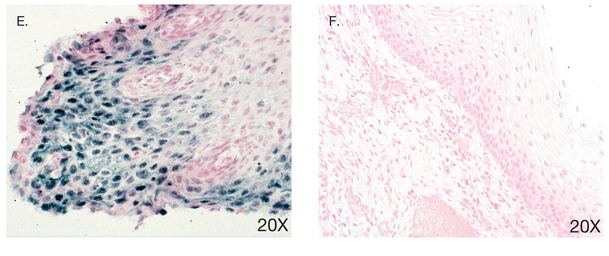




A. HPV high-risk — type 16, 18, 31, 33, 51 (red) detected with AMPIVIEW™ HPV High-Risk RNA probes in infected cervical tissue sample. B. No HPV detected with AMPIVIEW™ NSP-DIG probes (negative control).

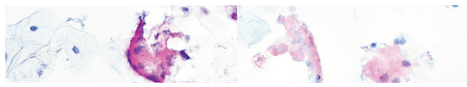


HPV low-risk (HPV 6, HPV 11) detected (blue) with AMPIVIEW™ HPV 6/11 RNA probes in C. infected cervical tissue sample and D. normal cervical tissue sample.



HPV high-risk (HPV 16, HPV 18) detected (blue) with AMPIVIEW™ HPV 16/18 RNA probes in E. infected cervical tissue sample and F. normal cervical tissue sample.

AMPIVIEW™ ISH vs PCR

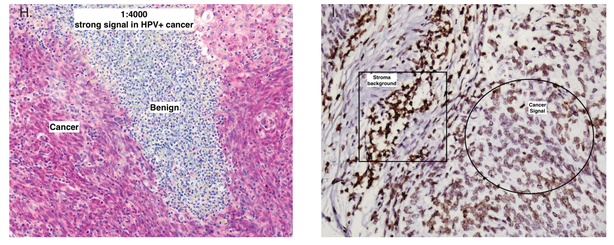


ISH Results	Negative	Positive	Positive	Positive
PCR Results	Negative	Positive	Positive	Positive
Ct Value*	ND	27.8	36.8	38.6

*Real-time PCR cycle threshold. Ct levels are inversely proportional to the amount of target nucleic acid in the sample.

Cells collected from pap smears were tested in parallel with ISH and RT-PCR to detect HPV high-risk genotypes. Results show that ISH detection is as sensitive as PCR detection.

High Specificity



H. HPV high-risk — type 16, 18, 31, 33, 51 (red) detected with AMPIVIEW™ HPV High-Risk RNA probes in infected cervical tissue sample. Note the lack if signal in the benign section of the tissue. I. Competitor's high-risk HPV probes were tested under the same conditions. Note the high signal in the stroma, where the virus should not be detected.

CONCLUSION

AMPIVIEW™ RNA probes are uniquely designed with the precision of targeted, sequence-specific RNA probes, powered by Enzo's LoopRNA ISH™ technology to deliver superior sensitivity and specificity. AMPIVIEW™ RNA probes sensitivity proved to be comparable to RT-PCR results, while preserving the morphoplogy of the sample. Additionally, the design of the probes make them adaptable to any workflow (manual or automated) and compatible with immunohistochemistry detection systems.

AMPIVIEW™ RNA probes are easy-to-use and adaptable with existing ISH and IHC setups. AMPIVIEW™ RNA probes can be designed to detect any gene and transcript of interest with virtually unlimited potential.

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