Spatial and temporal localization of nucleic acid targets in FFPE tissue samples with the novel LoopRNA™ technology

Deborah M. Holzapfel, PhD, Francesca Mazzoni, PhD, Jack Coleman, PhD

Enzo Life Sciences, Farmingdale, NY 11735

ABSTRACT

Spatial and temporal localization of DNA or RNA targets in formalin-fixed, paraffin-embedded (FFPE) cells and tissue samples can be achieved with *in situ* hybridization (ISH) technique, while preserving the morphology of the cell or tissue. ISH is used for the identification and localization of viral infections, analysis of transcriptionally active nucleic acid and its distribution in cells and tissues and identify sites of gene expression. While immunohistochemistry (IHC) shows protein localization in cells and tissue samples, ISH can help identify the cell of origin. In order to achieve successful results, we used AMPIVIEW™ ISH probes, which combines the precision of targeted, sequence-specific RNA probes with a superior sensitivity of loopRNA™ technology, making them compatible with nanopolymer detection systems used in IHC. This powerful tool can be designed to detect the nucleic acid expression patterns and spatial localization of individual targets or DNA-RNA, RNA-RNA interactions. In addition, RNA-protein interactions in tissues can be studied when combined ISH and IHC. This study explains how these probes are used not only for the detection of viruses such as SARS-CoV-2 and human papillomavirus (HPV), but also the expression of endogenous biomarkers such as HER2/neu in FFPE tissue samples.

INTRODUCTION

Spatial biology is the study of the tissue microenvironment, the ability to interrogate multiple genes and proteins in tissue samples in their original location (*in situ*), while keeping the tissue architecture and organization intact. This can be achieved with microscopy, various stains, antibodies and probes, which enables the scientists to assess every cell in a tissue sample, their products and location, the ability to determine cell-cell relationships, cell states and how each biomarker affect the its microenvironment.

Cells are the smallest structure capable of maintaining life. They compose all living things, from single-celled organisms to multibillion-celled animals. Cells can vary in type, size and shape, especially in the human body, which consists of 100 trillion cells, but they all perform the same basic life functions – use energy, respond to the environment and reproduce. A cell consists of three parts: the cell membrane, the nucleus and the cytoplasm (Figure 1A).

A tissue sample contains a group of cells that have similar structure and functions together as a unit (Figure 1B). There are four main tissue types in the body: epithelial, connective, muscle and nervous. Each tissue performs specific functions.

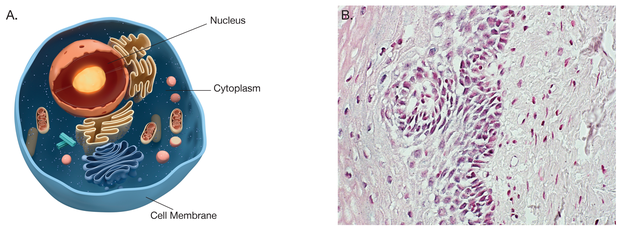


Figure 1 Cell vs Tissue
(A) A cell is comprised of a cell membrane, cytoplasm and nucleus. (B) A tissue is a group of cells that function together as a unit.

Protein is synthesized in cells using DNA, RNA and various enzymes. It generally includes transcription in the nucleus (DNA and RNA), translation (mRNA) and post-translational (protein) events in the cytoplasm. In order to detect the nucleic acids and proteins in the tissue samples, *in situ* hybridization (ISH) is used to detect DNA and RNA and immunohistochemistry (IHC) is used to detect proteins.

ISH is a method used for the precise spatial localization and detection of specific nucleic acid sequences (Figure 2A) in formalin-fixed, paraffin-embedded (FFPE) cells and tissue samples with DNA or RNA probes. IHC is a technique to stain and visualize protein markers in FFPE cells and tissues with antibodies against desired targets (Figure 2B). Both ISH and IHC are used to detect biomolecular markers (DNA, RNA or protein) without disrupting tissue morphology.

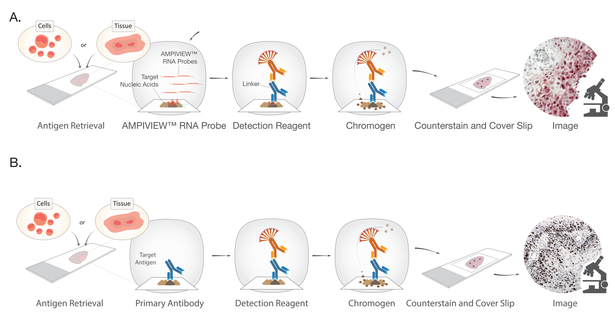


Figure 2 ISH vs IHC
(A) ISH workflow from antigen retrieval to imaging to detect nucleic acid targets in tissues and cells.
(B) IHC workflow from antigen retrieval to imaging to detect protein targets in tissues and cells..

This study introduces AMPIVIEW™ RNA ISH probes, which are designed with the precision of targeted, sequence-specific RNA probes, powered by Enzo's LoopRNA ISH™ technology to deliver superior sensitivity for the detection target genes.

METHODS

Immunohistochemistry (IHC) was performed manually with Enzo's antibodies and POLYVIEW® PLUS AP or HRP detection solutions and corresponding HIGHDEF® chromogens and counterstains according to manufacturer's instructions.

***in situ* Hybridization (ISH)** was performed with **AMPIVIEW™ RNA Probes,** POLYVIEW® PLUS HRP or AP detection reagents, DIGX® anti-digoxigenin linker, HIGHDEF® chromogens and counterstains according to manufacturer's instructions.

REFERENCES

1. Pardue, M.J; Gall, JG, "Molecular Hybridization of Radioactive DNA to the DNA of Cytological Preparations," PNAS, vol. 64, no. 2, pp. 600-604, 1969.
2. Wang , F, et. al., "RNAscope: a novel *in situ* RNA analysis platform for formalin-fixed, paraffin-embedded tissues.," Journal of Molecular Diagnosis, vol. 14, no. 1, pp. 22-29, 2021.
3. La Rocca, G, et al. "Recent improvements in *in situ* hybridization for the detection of HPV infections in clinical samples.," World Cancer Research Journal, 2020.
4. Nuovo GJ, et al. "A Standardization Protocol for the *In Situ* Detection of SARS-CoV2 RNA and Proteins." Appl Immunohistochem Mol Morphol. 2022 Feb 1;30(2):83-90.

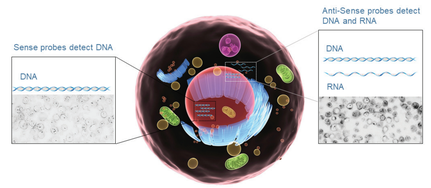
AMPIVIEW™ RNA Probes

Nucleic Acid Target

Select target nucleic acid (DNA or RNA) sequence from any species, any genome.

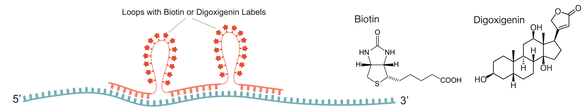
AMPIVIEW™ RNA Probes

Analyze sequence of the target nucleic acid and design the probes. Probes are produced with conjugated labels such as biotin or digoxigenin between complementary bases.



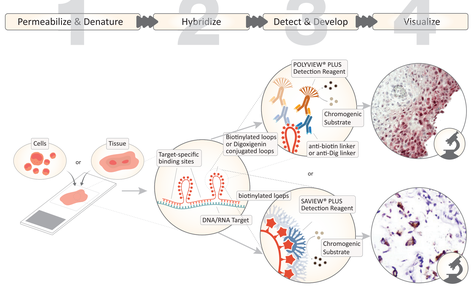
Probes can be designed as sense probes to detect DNA only or antisense probes to detect DNA and RNA.

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 IHC detection reagents.

AMPIVIEW™ ISH Workflow



SAVIEW® PLUS AP or HRP reagent can be used for biotinylated probes or POLYVIEW® PLUS AP or HRP can be combined with a linker. SAVIEW® PLUS or POLYVIEW® PLUS combined with corresponding HIGHDEF® chromogens delivers sharp and crisp staining.

RESULTS

High Specificity

AMPIVIEW™ RNA probes demonstrates high specificity with low background compared to competitor's product (Figure 3).

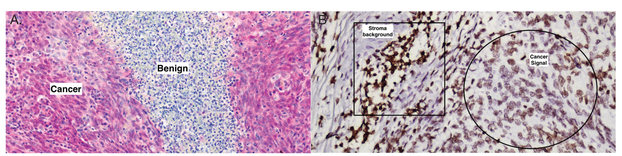


Figure 3. (A) HPV high-risk — type 16, 18, 31, 33, 51 (red) detected with AMPIVIEW™ HPV High-Risk RNA probes in cervical cancer tissue. (B) Competitor's high-risk HPV probes (brown) tested under the same conditions.

RESULTS

SENSE vs ANTISENSE PROBES

Signal comparison between sense probes (target DNA) and antisense probes (target DNA and RNA) showed that the antisense probes against HPV 16/18 yielded stronger signals in SiHa (1,3 copies of HPV) cells. Specificity was determined with the lack of detection of HPV 16/18 in C33 (no HPV copies) cells (Figure 4).

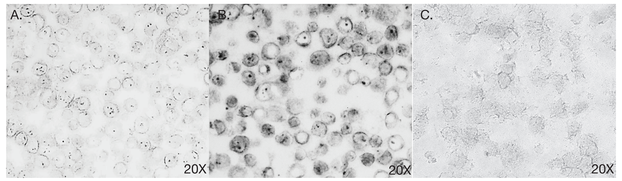


FIGURE 4. Detection of HPV 16/18 with (A) AMPIVIEW™ HPV 16/18 sense RNA probes, (B) AMPIVIEW™ HPV 16/18 antisense RNA probes in SiHa cells. (C) Detection of HPV 16/18 with AMPIVIEW™ HPV 16/18 antisense RNA probes in C33 cells.

HPV Detection

AMPIVIEW™ RNA Probes are specific for HPV 16/18/31/33/51, HPV 6/11 and HPV 16/18 genotypes. (Figure 5).

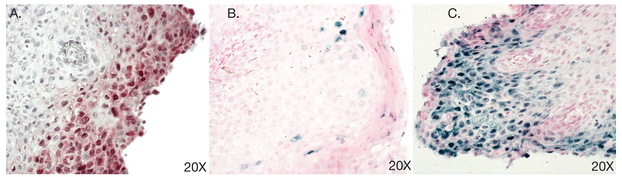


FIGURE 5. Detection of HPV with (A) AMPIVIEW™ HPV High-Risk RNA probes, (B) AMPIVIEW™ HPV 6/11 RNA probes and (C) AMPIVIEW™ HPV 16/18 RNA probes in HPV infected cervical tissues.

ISH vs IHC

Results obtained with AMPIVIEW™ HPV high-risk RNA probes were confirmed by the detection of p16 by IHC in cervical tissue infected with HPV. IHC and ISH can be combined for multiplexing (Figure 6).

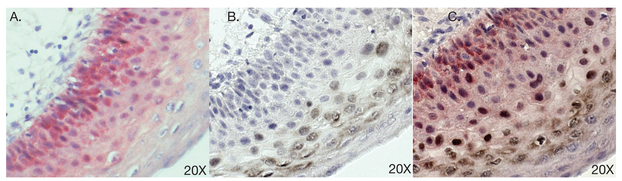


FIGURE 5. (A) Detection of p16 (red). (B) Detection of HPV with AMPIVIEW™ High-Risk HPV RNA probes (brown). (C) p16 and high-risk HPV detection with p16 antibody (red) and AMPIVIEW™ HPV High-Risk RNA probes (brown) in HPV infected cervical tissue.

CONCLUSION

AMPIVIEW™ RNA probes are carefully crafted, highly specific, powered by Enzo's LoopRNA ISH™ technology, to deliver superior sensitivity. The design of the probes make them adaptable to any workflow (manual or automated) and compatible with existing IHC detection systems and ISH setups. This innovation also enables the visualization of gene expression in the context of tissue architecture and analysis with a light microscope.

AMPIVIEW™ RNA probes can be designed to detect any gene and transcript of interest with virtually unlimited potential.