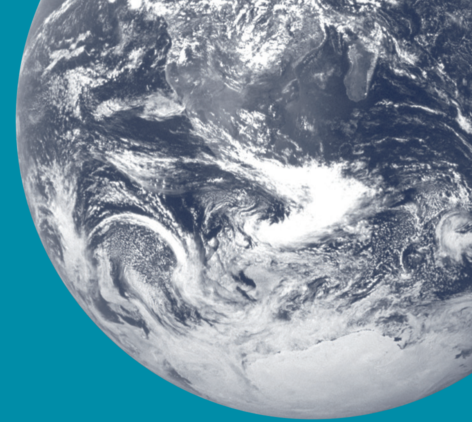

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LETTER TO THE EDITOR DETECTION OF HPV INFECTIONS IN CLINICAL SAMPLES: RECENT IMPROVEMENTS IN IN SITU HYBRIDIZATION (ISH) WITH LOOP RNA PROBES (LRPS) TECHNOLOGY"

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Dear Editor,

Cervical cancer is one of the most common cancers in women worldwide[1]. HPV infection may produce precancerous lesions which may progress to cervical cancer within 10-20 years after infection. So, early HPV infection detection can play an important role in reducing both frequency and mortality of the disease. To date, the International Agency for Research on Cancer (IARC) has detected 13 HPV genotypes as cervical carcinogens and, among them, HPV genotypes 16 and 18 are considered responsible for 50% of cervical intraepithelial neoplasia (i.e. CIN1 and 3) and for 70% of cervical cancer[2,3]. Moreover an active role of HPV has recently described in other district, such as oropharyngeal mucosa[4].

In Situ Hybridization (ISH) is a widely technique for the detection of nucleic acids species both in cells and tissues. Unlike sequencing and RT-PCR, the homogenization of the sample is not necessary with the consequent preservation of the information regarding cell and tissue morphology and target's subcellular localization. Currently, RNA scope technology (Advanced Cell Diagnostics-ACD) is considered the most sensitive and commercially available ISH technology for HPV RNA detection in cell lines and tissue sections; however, it requires considerable time and costs. To avoid these limitations, La Rocca et al[5] developed a novel ISH technology based on Loop RNA Probes (LRPs) with improved specificity and sensitivity and reduced time and costs. An important feature of LRPs is the inclusion of multiple RNA spacer segments, which are not complementary to the target nucleic acid sequence; so, when the probe is hybridized to the target sequence, the spacer segments loop out, facilitating the access of horseradish peroxidase (HRP) or alkaline phosphatase-conjugated antibodies. They compared LRPs technology with commercially available IHC kits for the HPV DNA and RNA detection, in cell lines, tissue samples and liquid-based cytology samples[5].

To this aim, two cell lines were employed: SiHa cells, which carry a defined number of integrated HPV 16 copies (1 to 3) per cell and C33A cells, not infected by HPV, were considered as negative control. The hybridization with an *antisense* HPV 16 LRP probe, showed in SiHa cells a punctate signal (representing the integration of the virus DNA genome in the host genome) and a diffuse signal (representing the HPV RNA locating within the cytoplasm); no signal was detected in C33A cells. The hybridization with a *sense* HPV 16 LRP probe, showed in SiHa cells only the punctate signal, demonstrating the ability of the technique to discriminate viral DNA integrated in the host genome from viral RNA. Finally, the treatment of the two cell lines with *RNase A* before the hybridization with the antisense HPV 16 LRP probe, induced

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a complete disappearance of the diffuse signal, confirming that LRPs can effectively detect HPV RNAs. Moreover, in SiHa cells, the hybridization with antisense HPV 16/18 LRP probe, showed a stronger signal than the hybridization of the same cell line with HPV 16/18 RNA scope reagents.

Similar results were obtained in Cervical Intraepithelial Neoplasia (CIN1) tissue section (FFPE biopsies): the staining with antisense HPV 16/18 LRP probe was more intense than HPV 16/18 double-stranded DNA (dsDNA) probes suggesting that LRPs are more sensitive than dsDNA probe.

Also liquid-based cytology samples, spread in monolayer, were incubated with antisense HPV (type 16, 18, 31, 33 and 51) LRP probes. The samples were previously analyzed by PCR and classified as high-grade squamous intraepithelial lesions (HSILs) and negative samples were included in the test to identify possible background signal. After hybridization, cell morphology was well-preserved and the HPV genome was detected in a very low number of infected cells.

In conclusion, the LRPs ISH technology is a very helpful instrument for HPV infection detection, by both manual and automated protocols, in cell lines, tissue samples and liquid-based cytology samples. It is a very easy-to-use and cost-effective method for the in situ detection of both low-copy HPV DNA and single copy integrated HPV RNA; it is a more sensitive, specific and rapid method than commercially available kits for HPV detection and its probes have a great thermodynamic stability when paired with both DNA and RNA target.

Conflict of Interest:
The authors declare no conflict of interest

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