109 AUTOMATED DETECTION OF HUMAN PAPILLOMA VIRUS (HPV) MRNA USING RNASCOPE TECHNOLOGY ON THE ONCORE PROX

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Background A detailed assessment of the biological status of cervical, head and neck, and condyloma tissues can be achieved by performing in situ hybridization using highly characterized probes to detect specific HPV E6/E7 mRNA. However, the manual test methods can be labor intensive. Applying commercial kits, such as RNAscope from ACD for mRNA detection, the basic manual protocol takes about 8 hours. Here we show the utility of the ONCORE ProX – an open, fully automated slide staining platform – for developing detection protocols for HPV mRNA using manual RNAscope kits.

Methods FFPE tissues: Cervical, head and neck, condylomas, positive for HPV infection. mRNA Probes from ACD or Ventana: HPV 6,11, 16,18 along with cocktails specific for low and high-risk types. Chromogens: DAB and Red used in the ACD and Ventana kits. RNA detection was performed following the main RNAscope protocol[1] for manual applications adapted for performance on the ONCORE ProX. The Ventana versions of the HPV mRNA detections were performed using their protocols and reagents on the Benchmark system.

Results The basic RNAscope protocol on the ONCORE ProX for detection of an HPV mRNA type takes 7–8 hours. We have developed protocols to identify HPV types typically seen in cervical (16,18) and head and neck (16) tissues and condylomas (6,11). The RNAscope chromogenic Brown and Red detections of low- and high-risk HPV were identical to the Ventana detection of the same types using their reagents. Suitable negative and positive control probes were included in these experiments, thus verifying the results for HPV staining on both platforms.

Conclusions In addition to the established functions for fully automated IHC and FISH, we have demonstrated the suitability of the ONCORE ProX for performing successfully and with relative ease complex technologies such as RNAscope to identify high and low risk HPV types in cervical, head and neck, and condyloma tissues. Although our work visually demonstrates similar staining detection capabilities when compared to the Ventana Benchmark, further work is required to tease out the subtle differences in detection of very low copy numbers of HPV subtypes.

REFERENCE

1. https://acdbio.com/manual-assays-rnascope

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