**Background** A detailed assessment of the biological status of tissues can be achieved by performing as many multiple target detections as possible on the same tissue slice of protein, DNA, and RNA. However, the tests methods, can be labor intensive. When 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 of mRNA using different RNAscope kits. Additionally, we developed protocols for the combined detection of Protein/mRNA and Protein/DNA/mRNA on the same slide.

**Methods** FFPE tissues used: Tonsil, breast MTA. Antibodies used: mouse IG kappa, rabbit ERBB2. FISH probe used: ERBB2/Copy Control 17 red/green in ZipFISH fast hybridization buffer. mRNA probes from ACD: IGK, ERBB2, CD3, CD20. RNA detection and visualization was performed following the main RNAscope protocols\(^1\) for manual applications adapted for performance on the ONCORE ProX. The basic RNAscope protocol for DAB detection was adapted for the combined detection of Protein/mRNA, and for detection of Protein/DNA/mRNA.

**Results** The RNAscope procedure\(^1\) involves steps present also in IHC and FISH protocols, thus we used this protocol as backbone for the development of protocols for the combined detection of protein and DNA targets. The basic RNAscope protocol for detection of a single mRNA type (e.g., ERBB2) takes 7–8 hours on the ONCORE ProX. We have additionally developed protocols for the sequential detection of protein and mRNA targets (IGK) and this takes about 9 hours. Furthermore, we detected on the same slide protein (ERBB2), DNA (ERBB2/copy control 17 FISH probe) and mRNA (ERBB2). This complex procedure takes about 11 hours, and it is possible thanks to ZipFISH buffer for FISH. Additionally, we have performed the RNAscope chromogenic detection of IGK mRNA in DAB and Red, as well as Chromogenic and Immunofluorescent Multiplex detection of CD3 and CD20 mRNAs.

**Conclusions** In addition to the established function for fully automated IHC, we have demonstrated the suitability of the ONCORE ProX for performing successfully and with relative ease, complex technologies such as RNAscope and furthermore the combined detection on the same slide of multiple targets such as protein (IF), DNA (ZipFISH) and RNA probes (RNAscope).

These features of the ONCORE ProX show its versatility and make it suitable for performing complex protocols to satisfy the needs of a rapidly developing field of molecular histology.

**REFERENCE**
