One Slide, All the Answers

An innovative RNA in situ hybridization (ISH) technology both localizes and quantifies RNA biomarkers, providing comprehensive diagnostic information on a single slide

Traditional ISH techniques rely on directly labeled nucleic acid probes and, consequently, may suffer from suboptimal sensitivity and specificity (limited ability to detect low-expression transcripts and relatively high levels of nonspecific staining). Advanced Cell Diagnostics (ACD, a Bio-Techne brand) RNA ISH – RNAscope™ – overcomes sensitivity issues by amplifying mRNA transcripts ~100-fold relative to traditional ISH technology. At the same time, it largely eliminates background noise by means of a paired probe approach. To learn more about this highly specific and sensitive system and its expanding diagnostic applications, we spoke to Dr. Rob Monroe, pathologist and Chief Medical Officer of Bio-Techne.

Superior detection, adaptable readout

RNAscope employs the proprietary Double-Z system, comprising Z-shaped paired probes in which the lower regions are complementary to target RNA and the upper regions consist of a 14-base tail sequence. Both probes must hybridize to contiguous target sequences for amplification to occur, greatly improving specificity. And, because a signal can be detected from only three Double-Z probe hybridizations, Bio-Techne's approach enables unambiguous identification of even very low-expression targets or fragmented mRNA transcripts common in formalin-fixed paraffin embedded (FFPE) tissue specimens. Notably, detection at a single-RNA molecular level is possible with the RNAscope signal amplification system. "The biomarker targets can be visualized with either chromogenic or fluorescent RNAscope detection kits according to user requirements," says Monroe.

Easy adoption

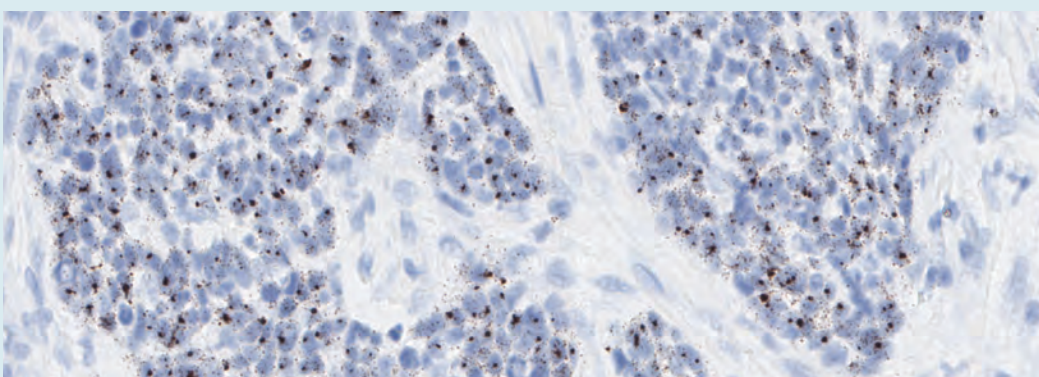
Monroe emphasizes the technology's user-friendliness, noting that no specific training or technical expertise is needed. "Lab technicians or researchers need only two ACD reagents: a target-specific probe and a detection kit," he says. Regarding the former, many users find what they need in ACD's catalogue of >30,000 probes spanning many different organisms and species. Failing that, says Monroe, submission of a GenBank target sequence will be used by ACD to design a sensitive and specific probe with an extremely low probability of cross-reacting with other targets.

The RNAscope procedure is straightforward and is ideally performed on an automated platform or with a hybridization oven to control slide temperature and humidity during amplification reactions. Unlike immunohistochemistry (IHC), RNAscope assay protocols are similar regardless of the target probe. Assay optimization steps are minimal relative to IHC antibody optimization, which requires testing various antibody clones, titers, and antigen retrieval methods. The technique's simplicity, reliability, and uniformity make it accessible to almost all clinical and research laboratories.

Meeting real-world needs

Monroe highlights RNAscope's compatibility with a broad range of sample types, including fresh frozen tissues and alcohol-fixed cytology specimens. "Most importantly, RNAscope is compatible with FFPE tissues – in fact, the system has been optimized for this type of sample." The assays are available for either manual or automated operation; compatible automation options include standard ISH staining platforms such as Leica BOND III, the Leica Bond Rx, and the Ventana Discovery instruments. This adaptability enables RNAscope to easily fit into existing IHC workflows and systems. Consequently, says Monroe, anatomic pathology labs can run RNAscope in parallel with normal IHC and provide outputs from both methodologies.

This flexibility makes RNAscope a pragmatic option; Monroe states that it is applicable to clinical diagnostics and translational pathology in a broad range of fields, including oncology, immuno-oncology, cell and gene therapy, neuroscience, and infectious disease. "It is of particular benefit for routine diagnostic pathology when IHC options are limited, for example where antibodies are unavailable or have poor specificity or low sensitivity," he says. In such cases RNAscope ISH often provides a better solution. "For example, IHC assessment of immunoglobulin expression in B cell lymphomas can be very challenging due to the low levels of kappa and lambda light chains, which are difficult to visualize over background; by contrast, the RNAscope readout comprises very discrete, punctate dots, simplifying the identification of cells



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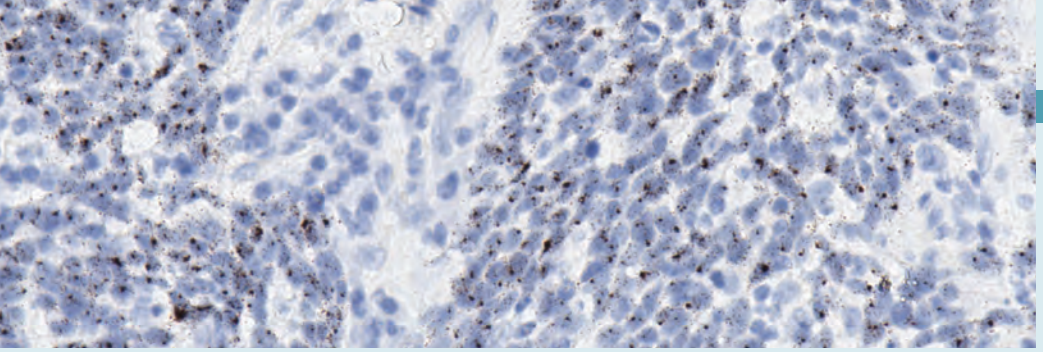

that express the gene of interest, such as immunoglobulin kappa or lambda in this case."

New insights in infectious disease

Ideal diagnostic reagents discriminate between related infectious agents – for example, between viruses in a given family (such as different SARS viruses). Does RNAscope ISH meet this ideal? Monroe is clear. "RNAscope can detect subtle differences based on nucleic acid changes and is therefore superior to IHC for infectious disease diagnostic applications." Furthermore, he adds, RNA ISH probes can be designed and produced quickly based on a target sequence. "Finding antibodies of similar discriminatory capacity is far more time-consuming and expensive."

In fact, says Monroe, RNAscope's utility in infectious disease diagnostics is already being realized, particularly for the detection of human papillomaviruses (HPV) in various clinical settings. "We now have RNAscope probes specific to almost all of the closely related HPV types," he states. "This permits elucidation of HPV's critical role in diseases such as head and neck cancer (HNC) and cervical dysplasia by providing the pathologist with a tool to both detect HPV and distinguish between types, including high- and low-risk, in biopsy samples." RNAscope is fast becoming the gold standard for the diagnosis of HPV-associated oropharyngeal squamous cell carcinoma (OPSCC), a unique subset of HNC associated with fewer recurrences, improved survival, and, in many cases, less aggressive treatment strategies. Relative to p16 IHC, a "surrogate" marker widely used for HPV assessment, the RNAscope test for high-risk HPV in OPSCC has similar high sensitivity but, importantly, superior specificity. Because it is not entirely specific for HPV, p16 IHC can be positive in ~10 percent of HPV-negative tumors. Such false positives are risky as they can affect treatment decisions and prognostic information provided to patients. RNAscope for high-risk HPV avoids these false-positives due to its direct detection of HPV.

Monroe also notes the technology's relevance to the COVID-19 pandemic. "Researchers put a lot of effort into developing diagnostic reagents for detection of the SARS-CoV-2 virus in nasopharyngeal samples, but paid less attention to the development of reagents for detection of the virus in situ to enable assessment of cellular targets and morphologic context, particularly in FFPE tissues." Did RNAscope ISH fill that gap? "Very effectively," says Monroe. "In many anatomic pathology labs, it has become the standard of care for assessing the presence of SARS-CoV-2 in autopsy tissues taken from patients, many of whom passed away before they could have a standard diagnostic test." Furthermore, RNAscope has been instrumental in highlighting the presence of SARS-CoV-2 in many cell types and organs affected by the virus, with over 100 publications on the virus to date using the technology. Going forward, ACD expects the RNAscope SARS-CoV-2 assay to be used broadly by anatomic pathology labs for the evaluation of various tissues suspected of involvement by the virus.

Beyond viral detection, RNAscope ISH probes are being used by pathologists for many oncology applications. One of the most useful RNAscope probes is albumin, a marker valuable in the evaluation of liver and biliary tumors. In particular, the RNAscope albumin assay helps pathologists in the diagnosis of intrahepatic cholangiocarcinoma versus extrahepatic cholangiocarcinoma and other liver tumors. Another emerging RNAscope application is detection of gene overexpression resulting from gene fusions or amplifications. The RNAscope assay for *MYB* is highly useful in the diagnosis of adenoid cystic carcinoma, with superior performance to FISH, the current gold standard. Similarly, the RNAscope *MDM2* assay is being used to distinguish benign lipomatous tumors from liposarcomas with similar performance to FISH.

Scope for the future

Novel technologies become routine over time, and RNAscope is expected to be no different. "In the near term, we are focused on making RNAscope technology and applications more widely available to help pathologists in their everyday diagnostic work. To realize this goal, automation of the technology on instruments like the BOND-III that are available in anatomic pathology labs around the world will be critical." ACD is also advancing the potential for RNAscope in the related field of companion diagnostics (CDx). Monroe notes, "We are working with a number of biopharma partners to develop RNAscope assays for predicting patient response to targeted therapies currently in clinical trials. We are optimistic that an RNAscope CDx assay will be approved in association with one of these therapeutics in the next several years."

In summary, RNAscope is an enabling technology that addresses diagnostic applications inaccessible to other approaches. It gives pathologists options for biomarker detection that traditional IHC cannot provide and is poised to become the standard of care for many targets and clinical applications. Monroe predicts that those who fail to adopt this approach in-house will, sooner or later, be compelled to send their samples to reference laboratories that offer RNAscope capabilities. ACD's vision, however, is that the simple, standardized nature of RNAscope protocols along with greater availability of automation will allow all laboratories to embrace this exciting technology. "Over time, we are confident that RNAscope will be a technique like IHC used by all anatomic pathology labs with dozens of compelling applications."