SUPERIOR ASSESSMENT OF IMMUNOGLOBULIN LIGHT CHAIN RESTRICTION WITH RNAscope™

RNAscope is a reliable and easy-to-interpret methodology for the detection of B-cell clonality in FFPE tissue, particularly when fresh tissue is not available for flow cytometry.

SPECIFIC AND SENSITIVE DETECTION OF B LYMPHOCYTE CLONALITY

Lymphoma is one of the most common cancers and, with early detection, has one of the highest survival rates. Clonal expansion of B lymphocytes harboring specific immunoglobulin gene rearrangements is a hallmark of B-cell lymphomas, resulting in expression of either immunoglobulin kappa or lambda light chains in combination with a specific immunoglobulin heavy chain.[1] This selective expression of immunoglobulin kappa or lambda is known as "light chain restriction". Evaluation of a population of lymphocytes for light chain restriction is an important component in the pathologic work-up and differential diagnosis which includes lymphoid hyperplasia, chronic inflammation, and lymphoma. Currently, flow cytometric analysis serves as the gold standard for evaluation of kappa and lambda light chain expression.[2] However, some B cell lymphomas lack sufficient surface immunoglobulin expression for detection by flow cytometry. In addition, fresh tissue is not always set aside or available for flow cytometry. In these cases, evaluation of light chain expression in formalin-fixed paraffin-embedded (FFPE) tissues is required. Several methods are available for FFPE evaluation, including immunohistochemistry (IHC) and conventional RNA *in situ* hybridization (ISH) but are often insufficiently sensitive to detect the lower levels of light chain expression present in B-cells relative to plasma cells.[2] PCR can also be performed on FFPE specimens for clonality assessment but consumes valuable tissue and precludes correlation with morphology in addition to requiring specialized molecular laboratories.

The RNAscope RNA ISH Platform, including chromogenic detection reagents and target specific probes for IgK and IgL, addresses the issues with IHC, conventional ISH, and PCR to provide clear and unequivocal assessment of light chain restriction in FFPE tissue (**FIGURE 1**). Typically, evaluation of a tissue specimen for light chain restriction involves performance of RNAscope for IgK and IgL independently on serial sections with brown chromogenic detection reagents (**FIGURE 2**). The combination of high sensitivity and specificity, morphological context, and compatibility with FFPE tissue sample types ensures straightforward identification of IgK and IgL expression even in the context of low levels of light chain mRNA expression that characterize most B cell lymphomas.

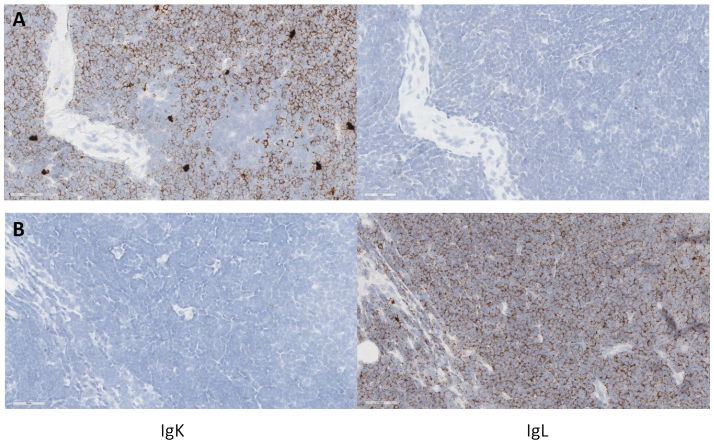

FIGURE 1. RNAScope RNA ISH evaluation of IgK and IgL expression. A. Example of B Cell lymphoma exhibiting IgK-restriction with RNAscope IgK and IgL probes; **B.** Example of B Cell lymphoma exhibiting IgL-restriction with RNAscope IgK and IgL probes

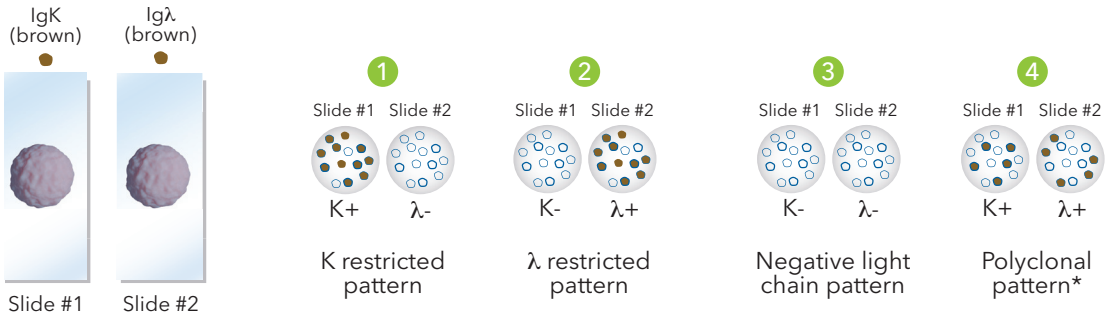

FIGURE 2. Interpretative Algorithm for Clonality Assessment with RNAscope IgK and IgL Probes and Brown Chromogenic Detection. Pattern 1 – IgK-restriction; Pattern 2 – IgL-restriction; Pattern 3 – Negative light chain expression; Pattern 4 – Polyclonal

In some cases, positivity is seen with both IgK and IgL RNAscope probes (**pattern 4, FIGURE 3**). This "polyclonal" pattern can be reflective of a non-clonal B cell proliferation such as lymphoid hyperplasia. However, this pattern can also be seen in approximately 10-20% of B cell lymphomas. If lymphoma is suspected based on other pathologic criteria, a third RNAscope probe for immunoglobulin lambda like peptide 5 (IGLL5) can be employed to further assess the apparent co-expression. IGLL5 is expressed during B cell development and in a subset of B cell lymphomas (**FIGURE 4**), regardless of clonality. IGLL5 shares some but not all sequences with the IgL constant region gene segments.[2,3] The IGLL5 RNAscope probe targets those sequences unique to the IGLL5 gene, preventing any cross-hybridization with IgL mRNA. However, the IgL probe targets a region of the IgL gene, the constant region, that overlaps with the IGLL5 gene and therefore has the potential to cross-hybridize with IGLL5 mRNA if present. As such, apparent co-expression of IgK and IgL with the RNAscope IgK and IgL probes can actually be due to the detection of IGLL5 expression by the IgL probe rather than expression of IgL. Use of the IGLL5 RNAscope probe for evaluation of cases with apparent co-expression of IgK and IgL is summarized in **FIGURE 5**.

Thus, the use of IgK, IgL and IGLL5 probes can help accurately detect B-cell clonality in multiple subtypes of B-cell lymphoma.

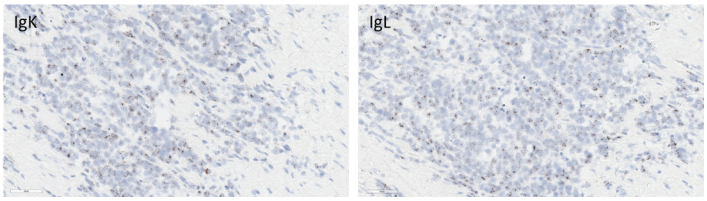

FIGURE 4. Polyclonal pattern of IgK/IgL co-expression.

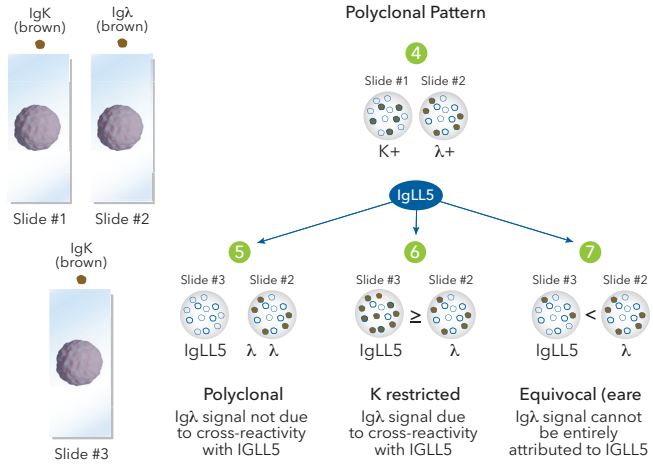

FIGURE 3. Interpretative Algorithm for Resolution of Polyclonal IgK+IgL Pattern. Pattern 5 (IgL>>IGLL5) Polyclonal, IgL signal not due to cross-reactivity withIGLL5; Pattern 6 (IGLL5>– IgL-restriction; Pattern 7 – Negative light chain expression; Pattern 4 – Polyclonal

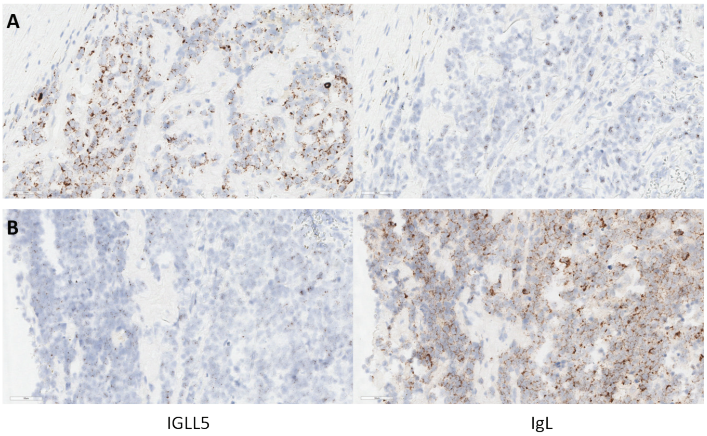

FIGURE 5. Resolution of Polyclonal IgK+IgL Pattern. A. IGLL5>>IGL, consistent with K restriction; IgL signal due to cross-reactivity with IGLL5. **B.** IGLL5<< IgL, equivocal for light chain restriction; IgL signal cannot be entirely attributed to IGLL5.

CONSISTENT, EASY-TO-INTERPRET RESULTS ENABLED BY AUTOMATION

The RNAscope *in situ* Hybridization Platform, including chromogenic detection reagents and target-specific probes like IgK/IgL, is a robust technology that allows for the identification of mRNA expression and localization at the single cell level with morphologic context in histologic sections. RNAscope is highly sensitive and specific due to its unique double Z probe design, resulting in an extremely high signal-to-noise ratio relative to traditional RNA ISH. The technology allows pathologists to visualize, localize, and quantify expression of a variety of RNA markers with an easy-to-interpret chromogenic format. RNAscope is readily available on automated platforms, including the Leica BOND III, for ease of use, and seamless integration into the anatomic pathology laboratory workflow.

REFERENCES

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2. Guo, L., Z. Wang, *et al*. (2018). "Ultrasensitive automated RNA *in situ* hybridization for kappa and lambda light chain mRNA detects B-cell clonality in tissue biopsies with performance comparable or superior to flow cytometry." Mod Pathol 31(3): 385-394.

3. Warford, A., M. Rahman, *et al*. (2019) "Pushing the boundaries of in situ hybridisation for mRNA demonstration: demonstration of kappa and lambda light chain restriction in follicular lymphoma." Br J Biomed Sci 76(3): 143-146.

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