In Situ Hybridization (ISH) – Novel techniques

Branched DNA ISH Technology

RNAscope/Basescope/ViewRNA

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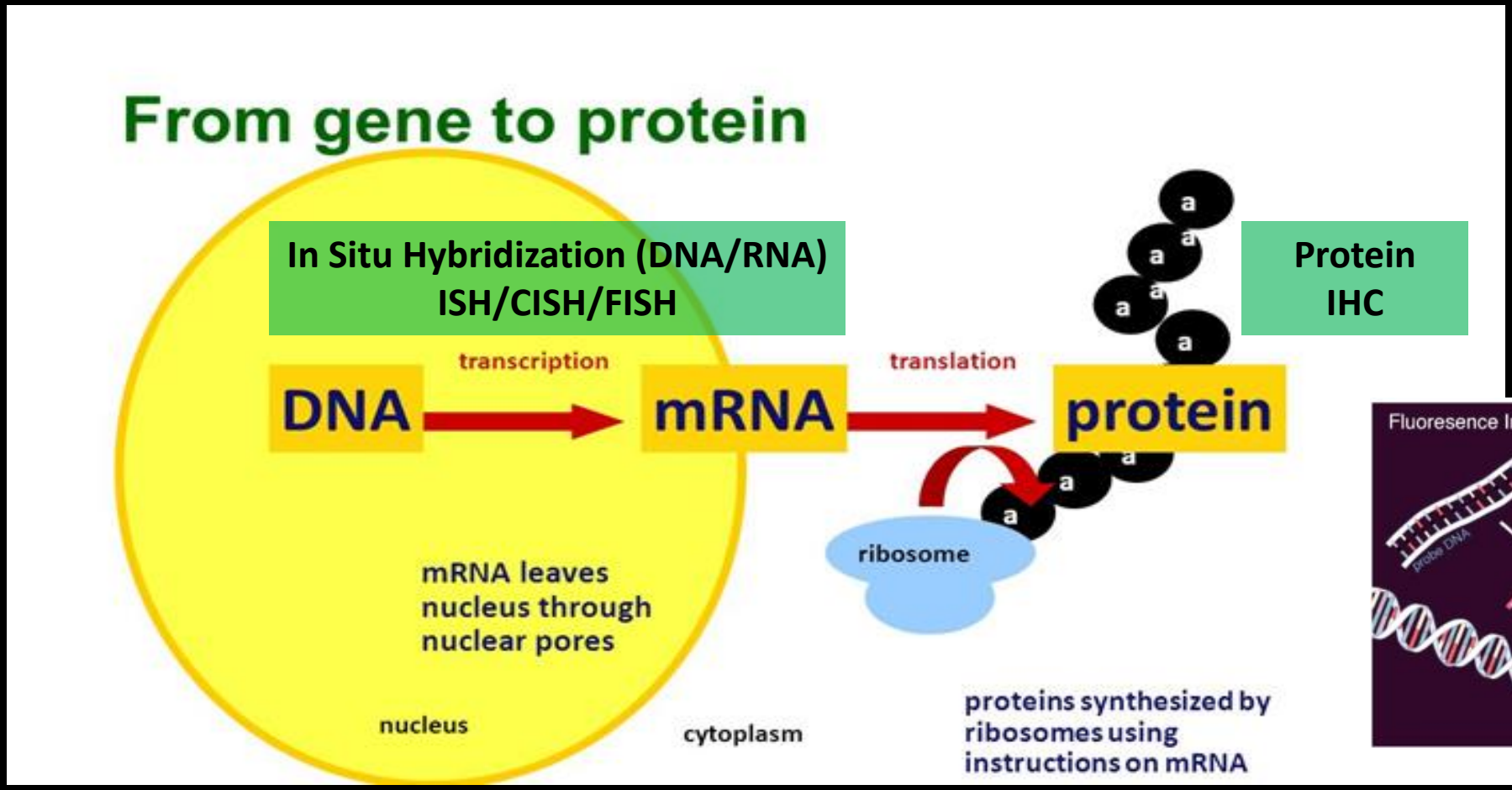
In Situ Hybridization (ISH)

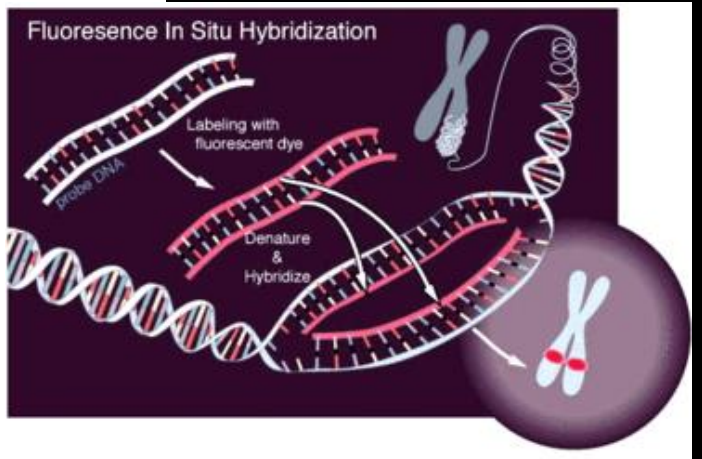
In situ hybridization (ISH) is a method using labeled complementary DNA, RNA or modified nucleic acids sequences (probes) annealing to specific target DNA or RNA molecules in cells or tissue sections.



Fluorescence (FISH)

Chromogenic (CISH)

……

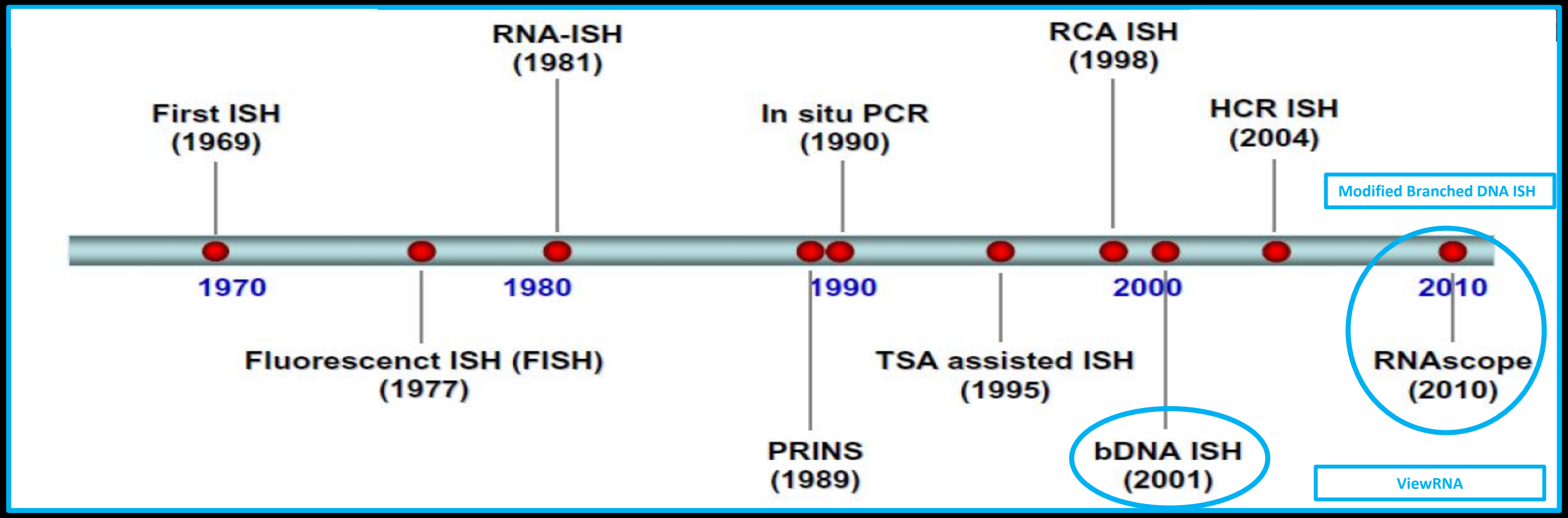


ISH: Typically use in the routine Pathology Department:

Chromogenic ISH (CISH)	Fluorescent ISH (FISH)	Research ISH (several techniques)
▪ Human Papilloma Virus (DNA) ▪ Epstein Barr Virus encoded RNA´s (EBER - small nuclear RNA) ▪ Cytomegalovirus (DNA) ▪ IGK/IGL (mRNA) ▪ HER-2/CEP17 (DNA) ▪ ……	▪ Foetal Pathology ▪ Haematology ▪ Carcinomas ▪ Sarcomas ▪ …… Numeric abnormalities (aneuploidy) Structural abnormalities Deletions e.g. del 17p13 (P53/CLL) Amplifications e.g.17q12 (HER2/Breast Ca.) Translocations e.g. t(9;22)(q34;q11) (CML) Inversions e.g. inv(2)(p21;p23) (ALK/EML4) ……	▪ mRNA **Base/RNA scope or ViewRNA** ▪ Long non coding RNA´s (LncRNA) ▪ Small non coding RNA`s (regulatory)  - mikro RNA (miRNA) - small nucleolar RNA`s (snoRNA) - small nuclear RNA´s (snRNA) - small-interfering RNA`s (siRNA) - PIWI-interacting RNA`s (piRNA) ▪ Other - e.g. circular RNA

LncRNA > 200 nucleotides
Regulatory small non coding RNA´s < 200 nuclotides

In Situ Hybridization (mRNA)



Conventional *in situ* RNA detection methodologies lack the sensitivity and specificity required to reliably detect rare or low-expressing RNA biomarkers within the tissue context

The Journal of Molecular Diagnostics, Vol. 14, No. 1, January 2012

DOI: 10.1016/j.jmoldx.2011.08.002

Technical Advance

RNAscope

A Novel in Situ *RNA Analysis Platform for Formalin-Fixed, Paraffin-Embedded Tissues*

Wang F et al.

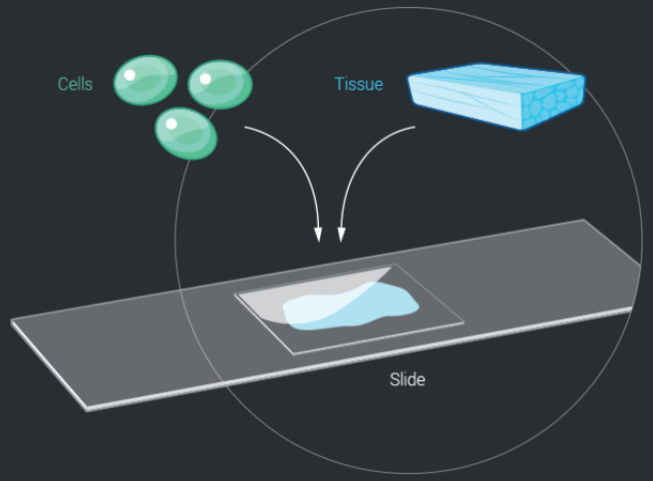
The first paper describing the use of Branched DNA ISH technology on formalin fixed and paraffin embedded tissue.

RNAscope® In Situ Hybridization Assay Workflow

Step 1:

Permeabilize

Tissue sections or cells are fixed onto slides and pretreated with RNAscope® Pretreatment Kit to unmask target RNA and permeabilize cells.

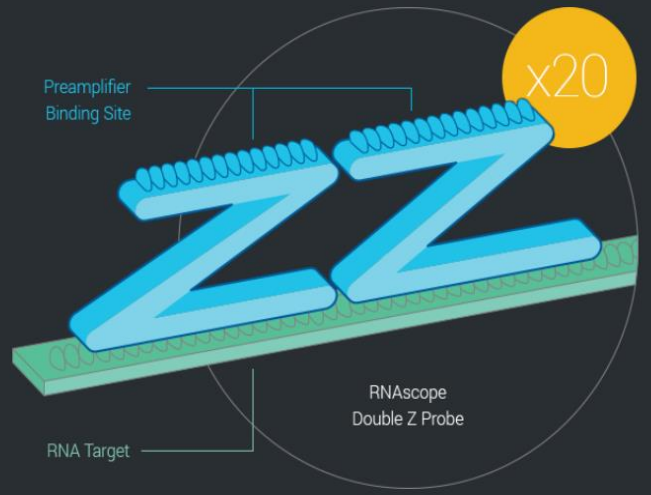


RNAscope® In Situ Hybridization Assay Workflow

Step 2:

Hybridize

Designed with ~20 target-specific double Z probes, RNAscope® Probes hybridize to target RNA molecules.



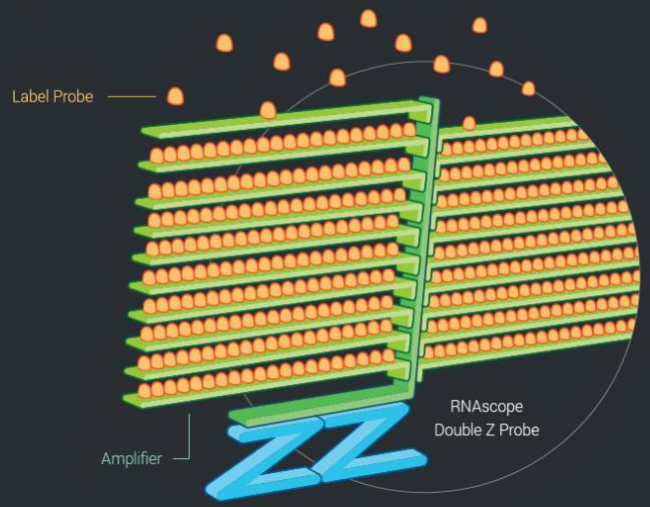
Modified Branched DNA ISH

RNAscope® In Situ Hybridization Assay Workflow

Step 3:

Amplify

RNAscope® Detection Reagents amplify the hybridization signals via sequential hybridization of amplifiers and label probes.



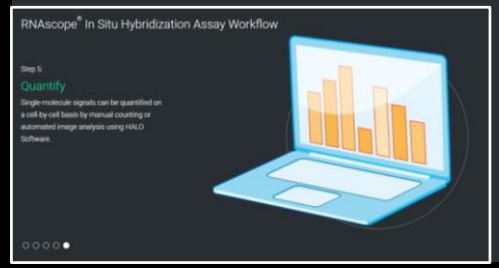
Modified Branched DNA ISH

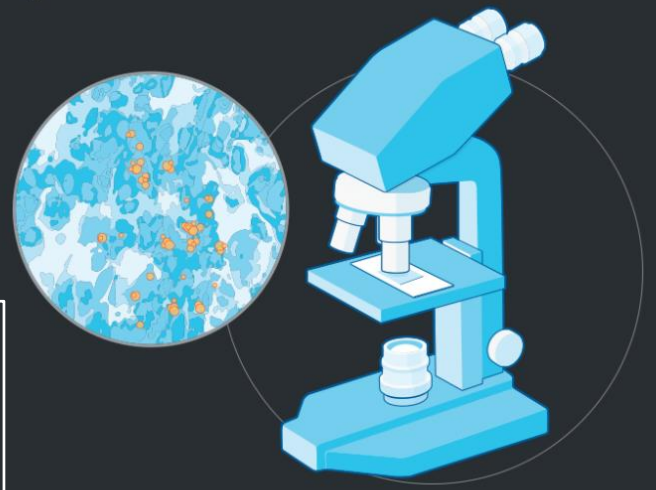
RNAscope® In Situ Hybridization Assay Workflow

Step 4:

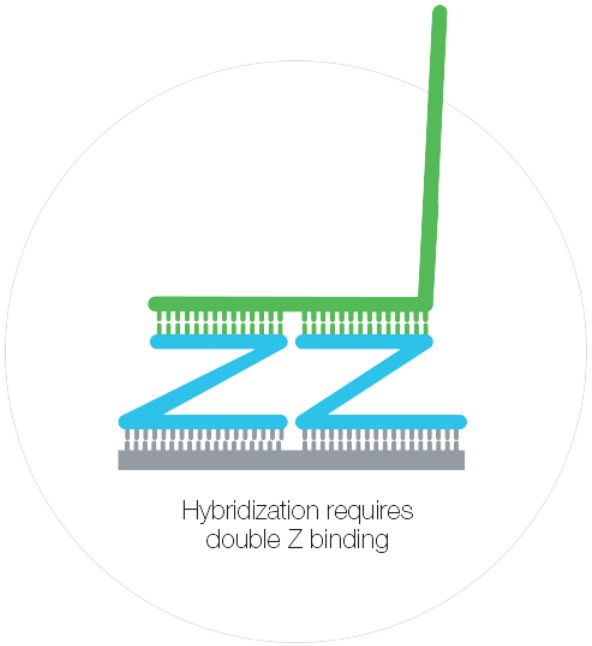
Visualize

Each punctate dot signal represents a single test target RNA molecule and can be visualized with a microscope.





SinglePlex /Advance Cell Diagnostic (ACD)



RNAscope® Probe Design and Signal Amplification Strategy

In order to substantially improve the signal-to-noise ratio of RNA ISH, RNAscope® employs a probe design strategy much akin to fluorescence resonance energy transfer (FRET), in which two independent probes (double Z probes) have to hybridize to the target sequence in tandem in order for signal amplification to occur. As it is highly unlikely that two independent probes will hybridize to a non-specific target right next to each other, this design concept ensures selective amplification of target-specific signals.

For each target RNA species, ~20 double Z target probe pairs are designed to specifically hybridize to the target molecule, but not to non-targeted molecules.

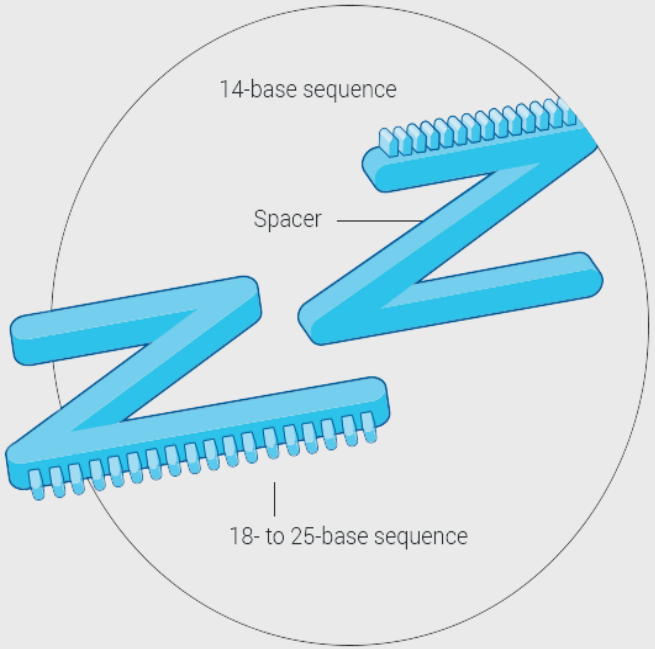
- > 14000 target probes.
- New customer probes in two weeks (development/manufacturing).

Each Target Z Probe Contains Three Elements

The lower region of the Z is an 18-to 25-base region that is complementary to the target RNA. This sequence is selected for target specific hybridization and uniform hybridization properties.

A spacer sequence that links the two components of the probe. The upper region of the Z is a 14-base tail sequence.

The two tails from a double Z probe pair forms a 28 base binding site for the pre-amplifier.



BaseScope vs RNAscope (mRNA ISH)

	BaseScope	RNAscope
Size of target RNA	▪ RNA 50-300 nt (bases)	▪ mRNA > 300 nt ▪ lncRNA > 300 nt
Number of ZZ pairs pr. target	▪ 1-4 ZZ pairs depending on application	▪ Standard 20 ZZ pairs (minimum 6 ZZ pairs)
Application	▪ Single RNA molecule detection ▪ Exon Junction/splice variants, point mutation and short RNA sequences ▪ Other (e.g., gene fusion)	▪ Single RNA molecule detection
Detection options	▪ Single (Chromogenic Red) ▪ Duplex (Chromogenic Green/Red)	▪ Single (Chromogenic Brown or Red) ▪ Duplex (Chromogenic Green/Red) ▪ Multiplex Fluorescent (up to 3 RNA targets) ▪ Hiplex Fluorescent (up to 8 RNA targets)
Automation	▪ None (only manual assays)	▪ Bond Rx (Leica): Single, Duplex and Multiplex ▪ Ventana Discovery(Roche): Single and Duplex
Workflow length	▪ 8.5 Hours (Single Staining)	▪ 8 Hours (Manual/Single staining)
Probes	▪ C1 (HRP)/C2 (AP) Channels	▪ C1/C2/C3/C4…… ?

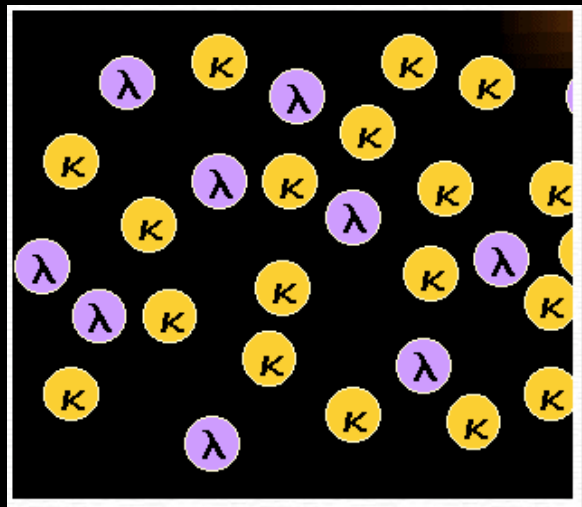
Long non-coding RNAs (lncRNAs) are a large and diverse class of transcribed RNA molecules with a length of more than 200 nucleotides that do not encode proteins (or lack > 100 amino acid open reading frame). lncRNAs are important regulators of gene expression, and lncRNAs are thought to have a wide range of functions in cellular and developmental processes.

Our approach to mRNA ISH (RNAscope)

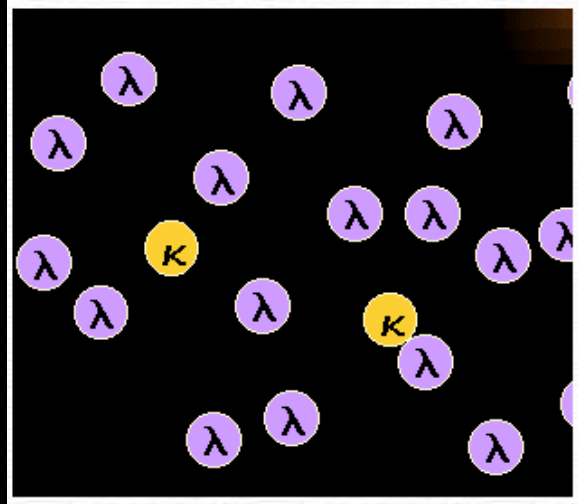
- Helpful in challenging diagnostic situations e.g., detection of light chain restrictions (kappa/lambda) in B-cell Lymphomas
- Confirming mRNA findings (e.g., Nanostring profiling) – which cells are positive
- Validation/verification of reaction patterns obtained with research antibodies
- Lack of valid primary antibodies
- BaseScope

e.g. point mutation (BRAF V600E in melanomas or colon adenocarcinomas) or gene fusion products

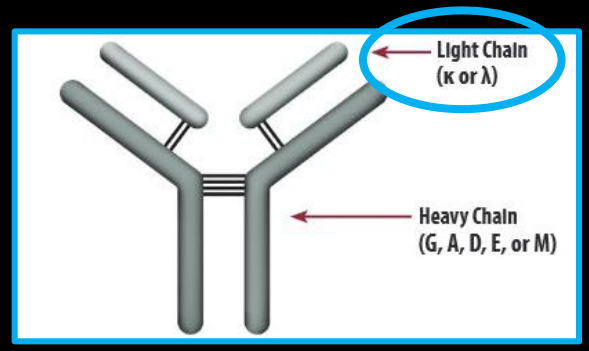
B-cell lymphomas and plasma cell disorders are characterized by showing immunoglobulin light chain restrictions and is the hallmark of discriminating reactive conditions from malignant transformation .



Normal, polyclonal B-cells are a mixture of kappa-B-cells and lambda-B-cells.
Our B-cells have a special feature letting us detect their clonality easily: A B-cell carries either kappa- or lambda-light chains on its surface. And normal polyclonal B-cells are a mixture of kappa-B-cells and lambda B-cells as can be seen in the left-hand figure.



Monoclonal mature B-cells are either kappa or lambda.
If a malignant B-cell clone proliferates this will result in a B-cell population consisting of either only kappa- or only lambda-B-cells. The latter case (i.e. lambda-monoclonal B-cells) is symbolized in the left-hand figure.
Note: in rare cases we find no light chain expressed on the B-cell surface, even in a mature B-cell neoplasm. This makes the diagnosis a little bit more difficult.



In general:

B-cells express membranous Ig`s

Plasma cells express cytoplasmic Ig´s and secret Ig`s to the surrounding tissue

Demonstrations of immunoglobulin light chain restrictions in mature B-cell lymphomas

Challenges:

- **Fresh and unfixed material unavailable for Flowcytometric investigations (Standard method).**
  - Kappa/lambda antibodies are used in panels with other hematolymphoid markers
- **Immunohistochemistry have the tendency to be confounded by background staining.**
  - Serum immunoglobulin
  - Require carefully calibrated protocol (difficult) and “optimal” pre-analytic conditions
  - Risk of false positive results due suboptimal fixation
- **Lack of sensitive and robust mRNA ISH technology for FFPE tissue**
  - Mature B-cell lymphomas often express low level of membranous immunoglobulin (protein) and thus, low level of mRNA K/L

Rimsza et al. Diagnostic Pathology 2014, 9:144
http://www.diagnosticpathology.org/content/9/1/144

DIAGNOSTIC PATHOLOGY

METHODOLOGY Open Access

Kappa and lambda light chain mRNA in situ hybridization compared to flow cytometry and immunohistochemistry in B cell lymphomas

Lisa M Rimsza[1*], William A Day[2], Sarah McGinn[1], Anne Pedata[2], Yasodha Natkunam[3], Roger Warnke[3], James R Cook[4], Teresa Marafioti[5] and Thomas M Grogan[2]

mRNA Kappa or Lambda light chain

Demonstration of monoclonality in B-cell proliferations using mRNA CISH standard procedures, is most often useful in myeloma and cases with plasmacytic differentiation due to high mRNA level (Kappa or Lambda) in these disorders.

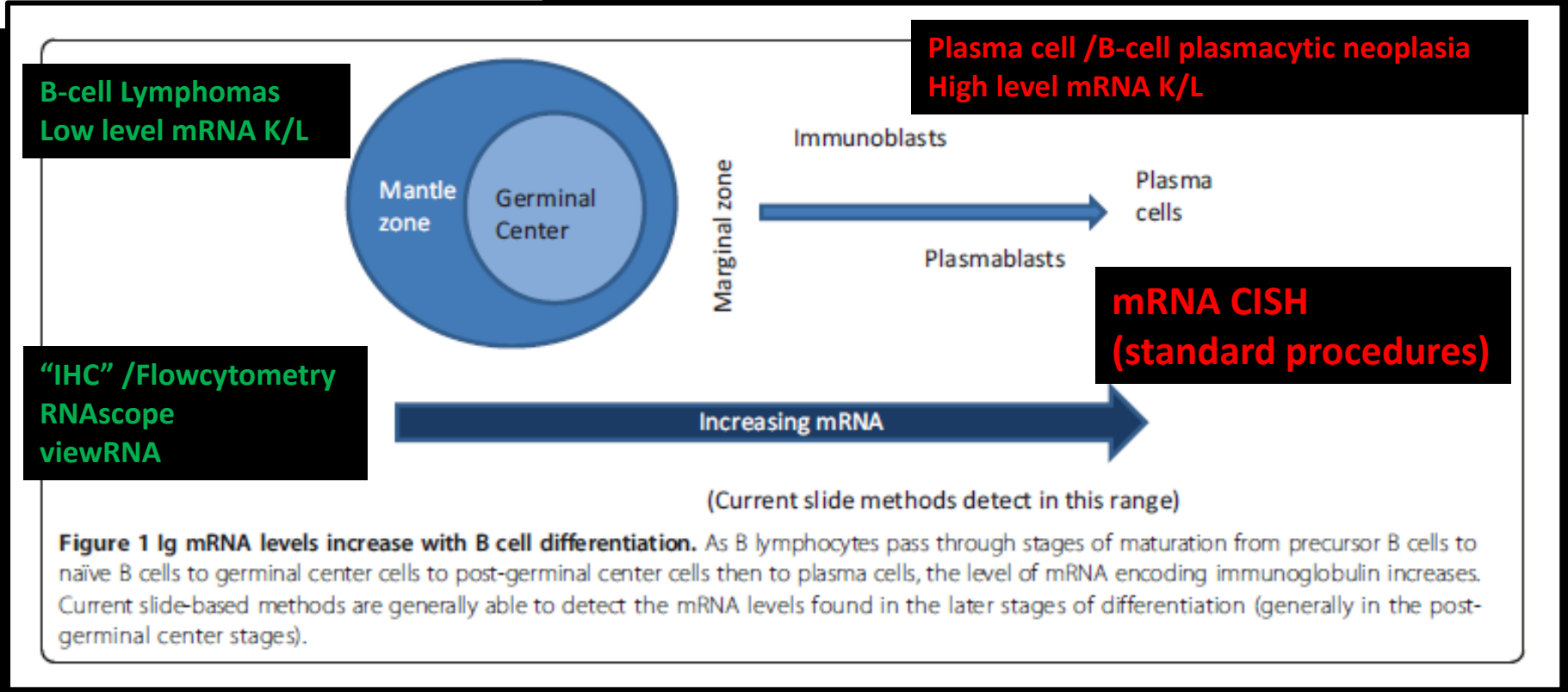


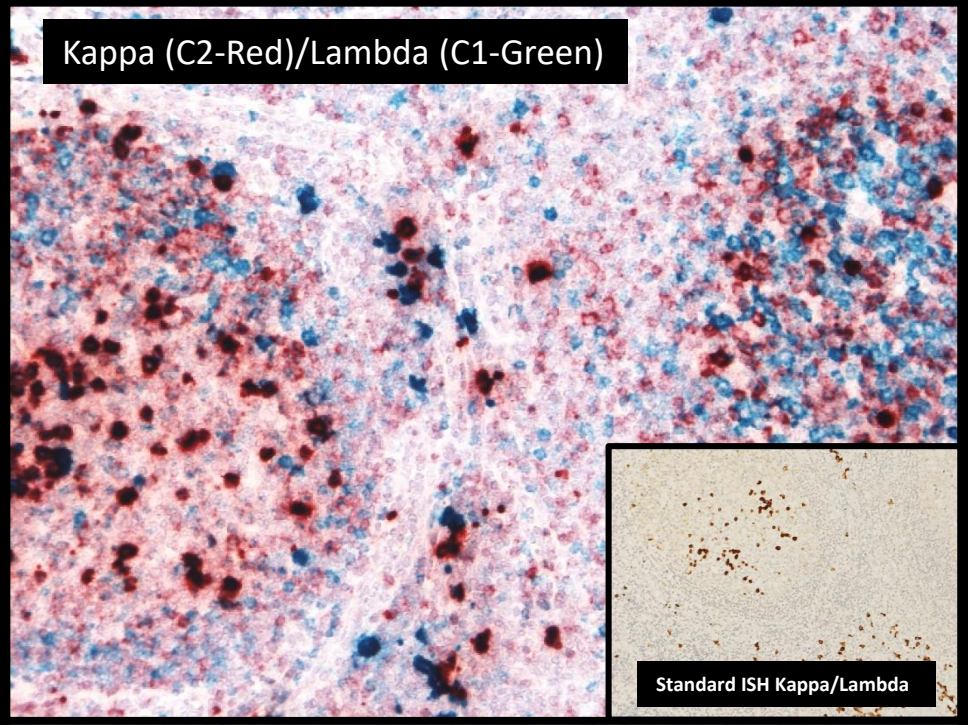
Figure 1 Ig mRNA levels increase with B cell differentiation. As B lymphocytes pass through stages of maturation from precursor B cells to naïve B cells to germinal center cells to post-germinal center cells then to plasma cells, the level of mRNA encoding immunoglobulin increases. Current slide-based methods are generally able to detect the mRNA levels found in the later stages of differentiation (generally in the post-germinal center stages).

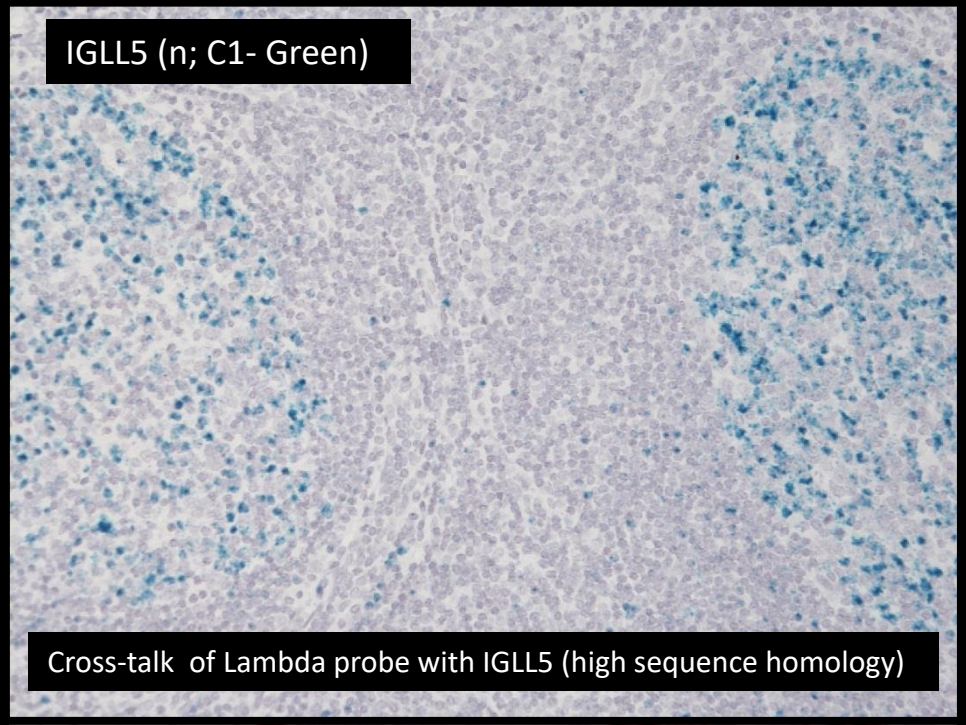
Test: RNAscope for light chain restriction (Kappa/Lambda)

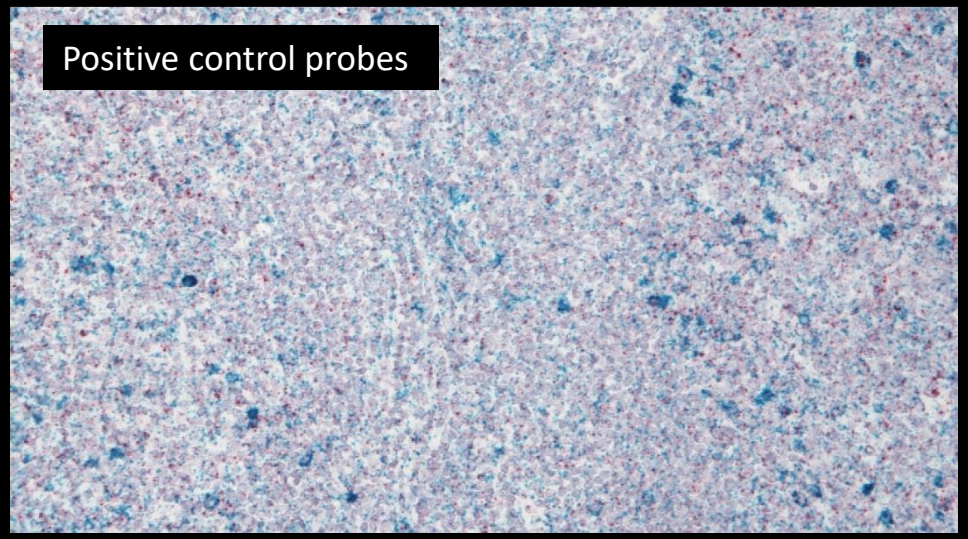
TMA`s /Diagnosis	Clinical info Light chain restriction
Lymphoplasmacytoid lymphoma (LPL) (1) Næ	Lambda^{+}/Kappa^{-} (IHC^{+}/ISH^{+})
Lymphoplasmacytoid lymphoma (LPL) (2)/Næ	Lambda^{+}/Kappa^{-} (IHC^{+}/ISH^{+})
Lymphoplasmacytoid lymphoma (LPL) (3)/Næ	Kappa^{+}/Lambda^{-} (IHC^{+}/ISH^{+})
Lymphoplasmacytoid lymphoma (LPL) (4)/Ros	Kappa^{+}/Lambda^{-} (FC)
Lymphoplasmacytoid lymphoma (LPL) (5)/Ros	Kappa^{+}/Lambda^{-} (FC)
Lymphoplasmacytoid lymphoma (LPL) (6)/Ros	Kappa^{+}/Lambda^{-} (FC)
Lymphoplasmacytoid lymphoma (LPL) (7)/Ros	Unknown
Lymphoplasmacytoid lymphoma (LPL) (8)/Ros	Unknown
Myeloma/Næ	Kappa^{+}/Lambda^{-} (IHC^{+}/ISH^{+})
Mantle cell lymphoma (MCL) (1)/Næ	Kappa^{+}/Lambda^{-} (IHC^{+}/ISH^{-})
Mantle cell lymphoma (MCL) (2)/Næ	Kappa^{+}/Lambda^{-} (IHC^{+}/ISH^{-})
Mantle cell lymphoma (MCL) (3)/Næ	Lambda^{+}/Kappa^{-} (IHC^{+}/ISH^{-})
Mantle cell lymphoma (MCL) (4)/Næ	Lambda^{+}/Kappa^{-} (IHC^{+}/ISH^{-})
Follicular Lymphoma (FL) (1)/Ros	Kappa^{+}/Lambda^{-} (FC)
Follicular Lymphoma (FL) (2)/Ros	Unknown
Follicular Lymphoma (FL) (3)/Ros	Kappa^{+}/Lambda^{-} (FC)
Diffuse Large B-Cell Lymphoma (DLBCL) (1)/Ros	Lambda^{+}/Kappa^{-} (FC)
Diffuse Large B-Cell Lymphoma (DLBCL) (2)/Ros	Unknown
Tonsil (Fix time 6-168h)	Poly
Negative control tissue (Appendix, Kidney and placenta)	Negative

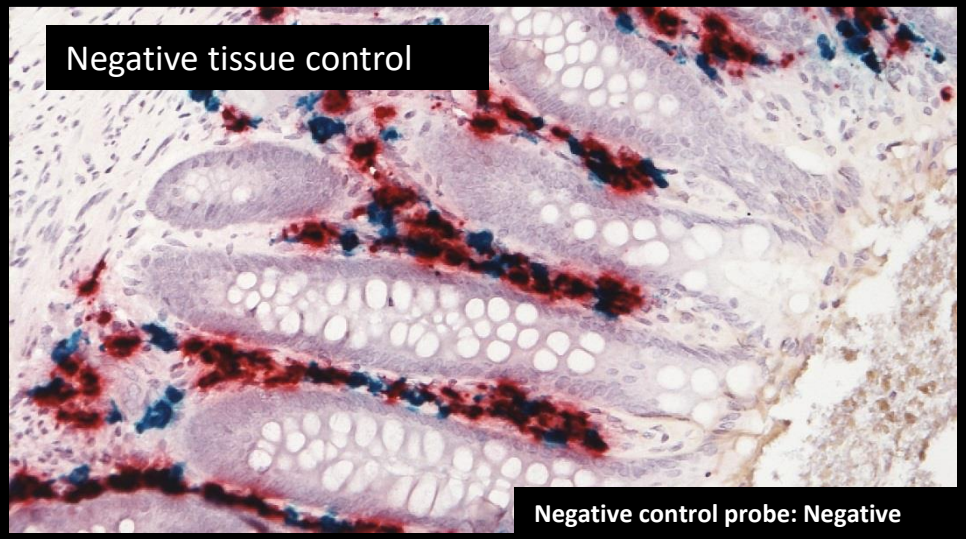
RNA Scope Duplex

Tonsil fix 96h (NBF)









IGLL5 = Immunoglobulin Lambda Like Polypeptid 5

(H15/P10-20-30)

Mantle cell B-cell Lymphomas

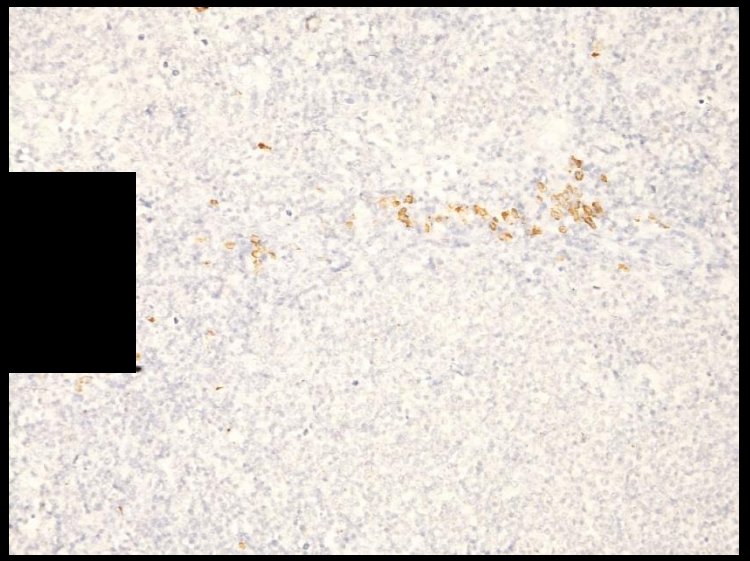
Standard ISH (Kappa)/DAB

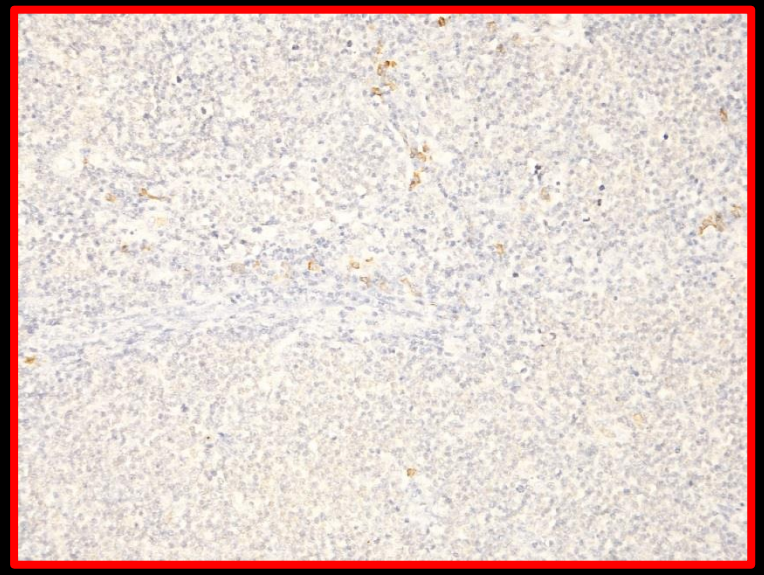
Standard ISH (Lambda)/DAB

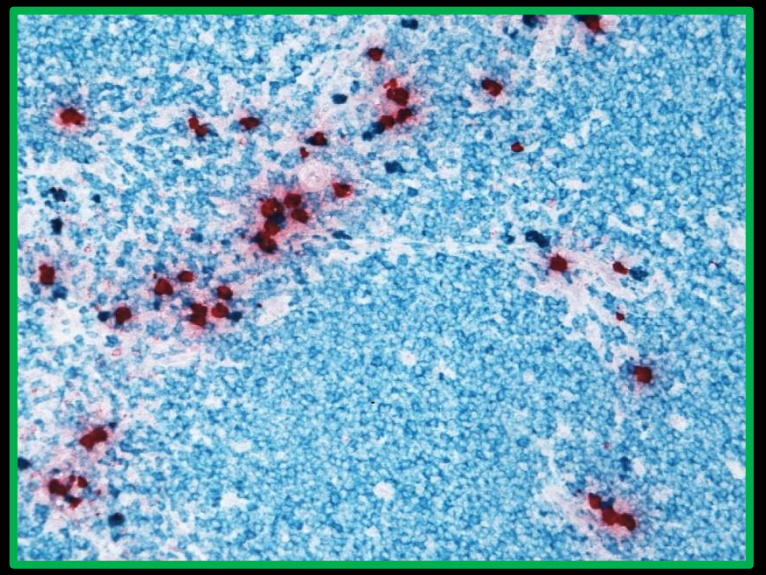
RNAscope Kappa (C2-Red)/Lambda(C1-Green)

Mantle cell Lymphoma

Lambda +

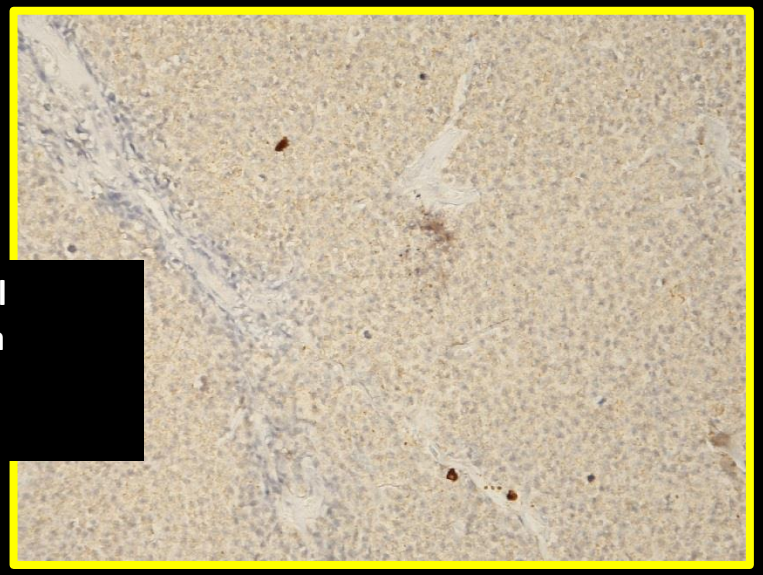


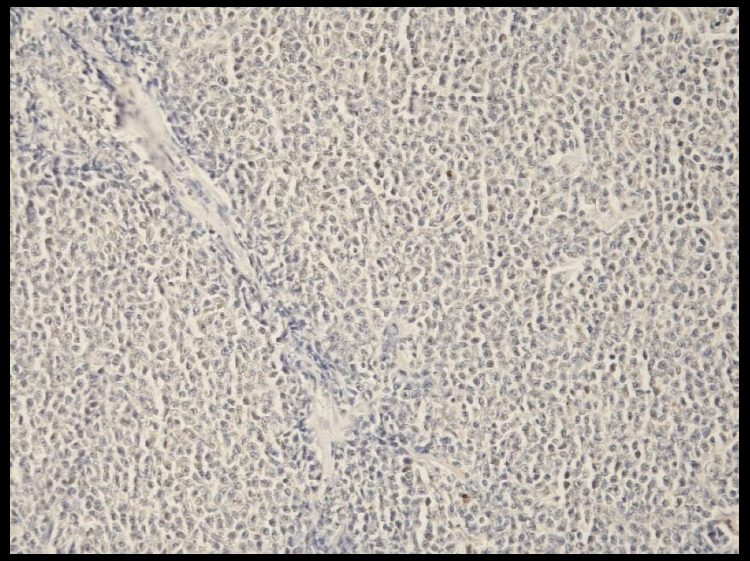


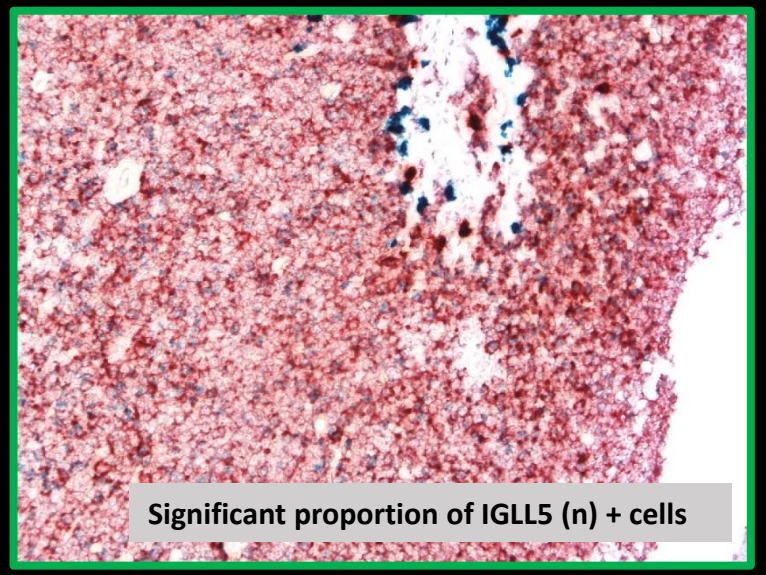


Mantle cell Lymphoma

Kappa +





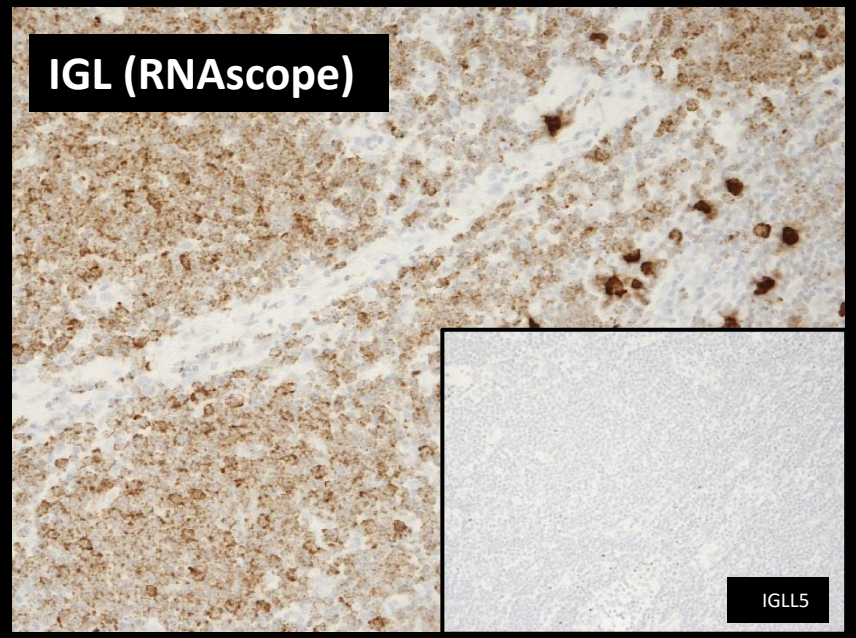


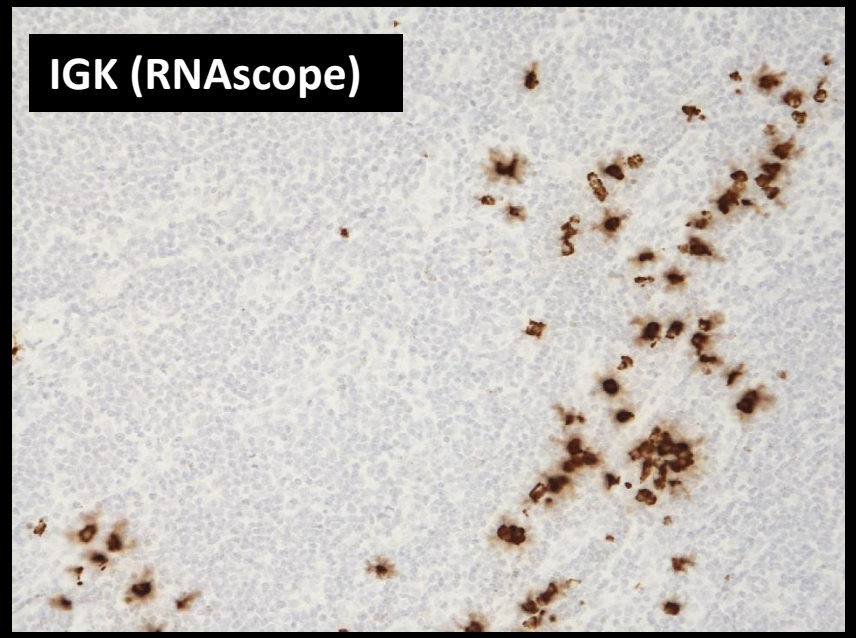
Mantle cell B-cell Lymphomas

RNAscope (SinglePlex/DAB) K/L

Mantle cell Lymphoma

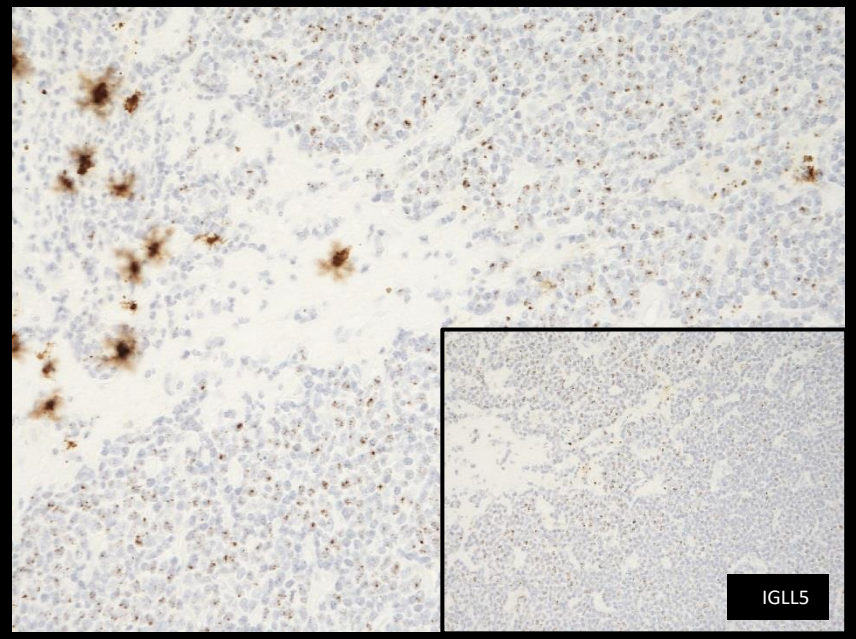
Lambda +

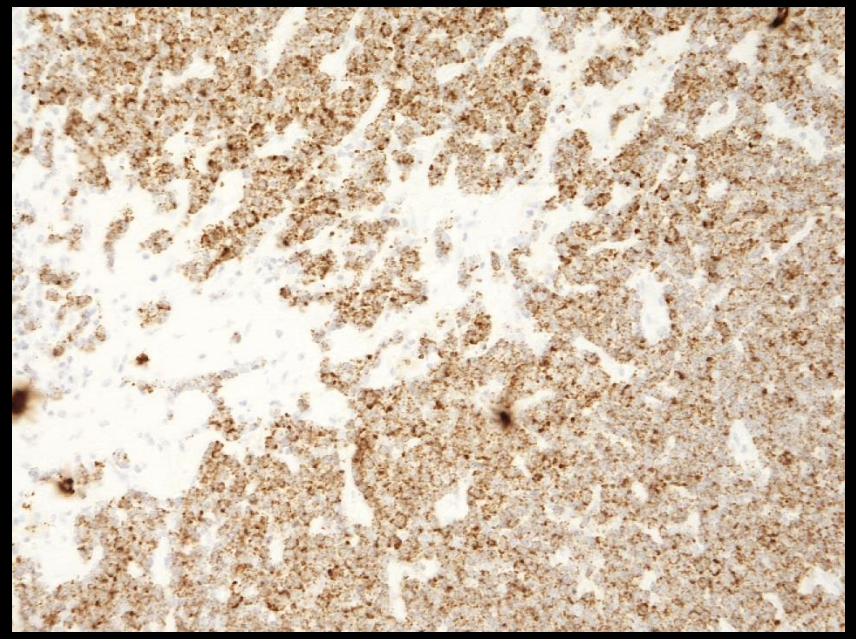




Mantle cell Lymphoma

Kappa +



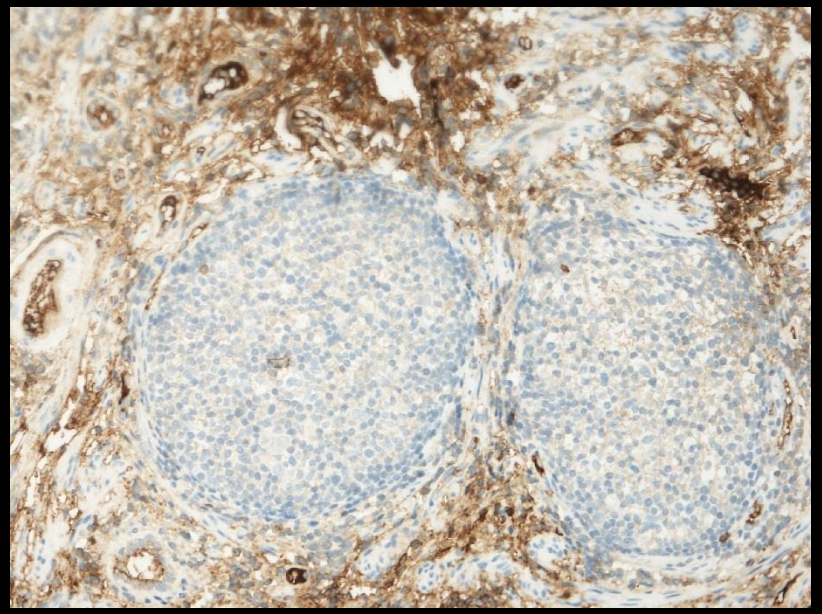


Low grade B-cell Lymphoma

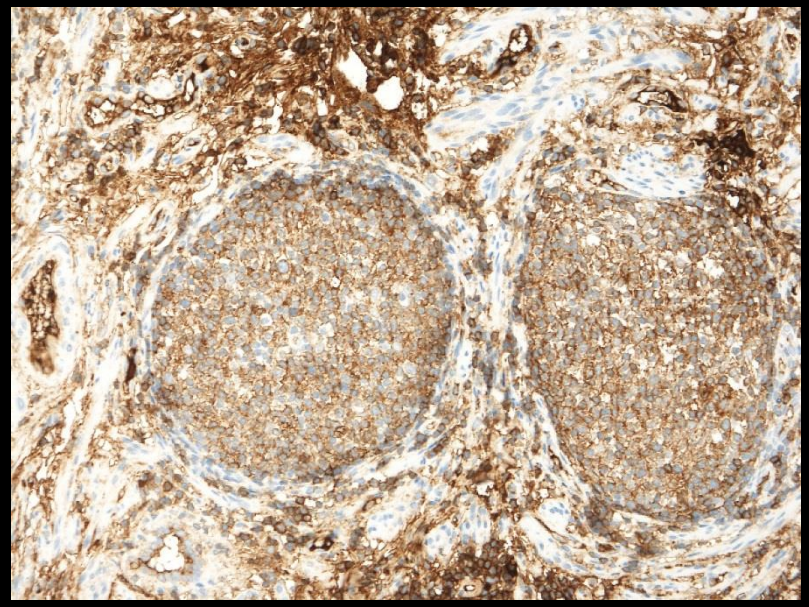
RNA Scope (Duplex)

Follicular Lymphoma (2)/Ovary

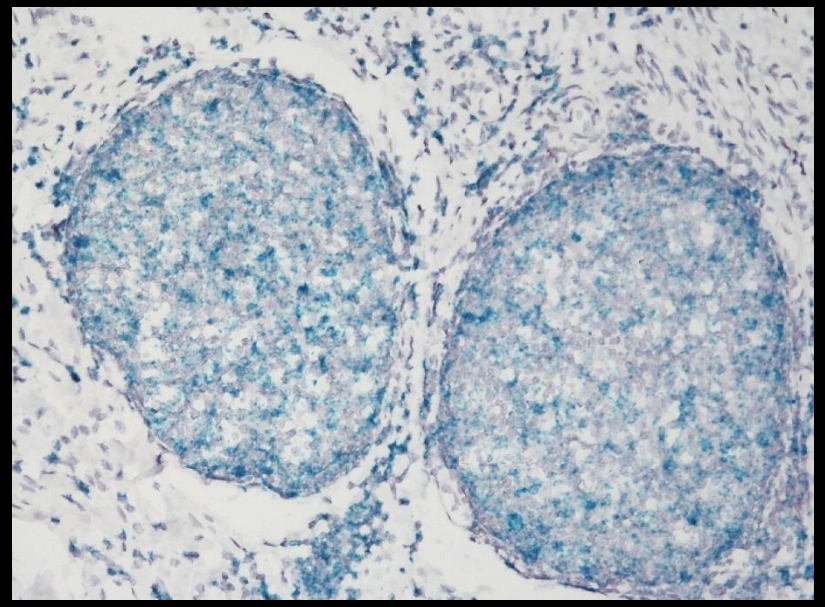
Clinical info: Unknown Kappa/Lambda status (Ros)



IHC Kappa (re-test)



IHC Lambda (re-test)



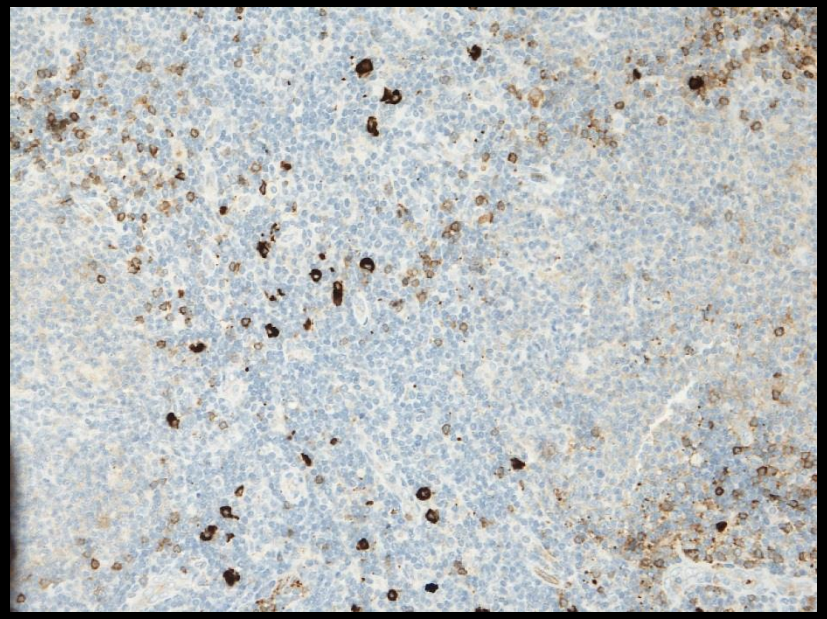
RNA scope Kappa(C2-Red)/Lambda (C1-Green)

(H15/P10-20-30)

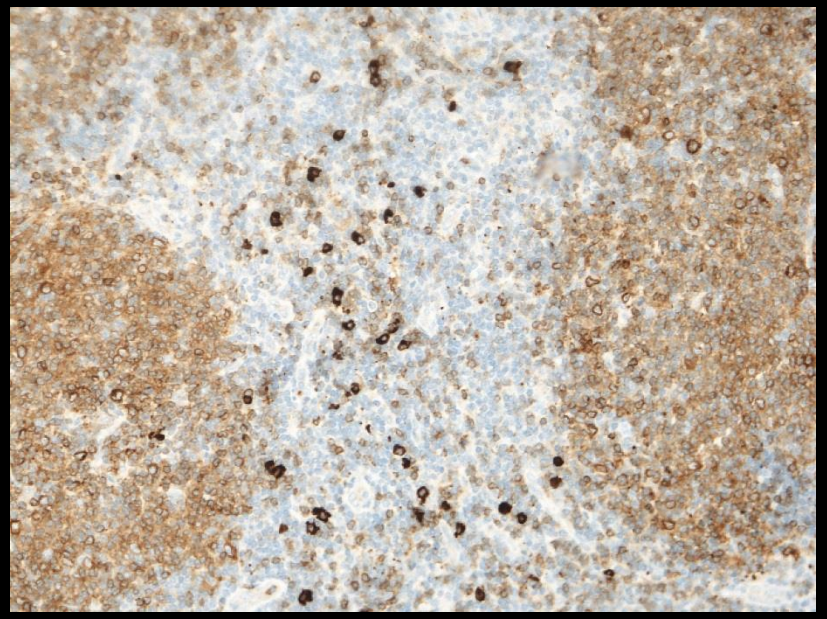
Low grade B-cell Lymphoma
RNA Scope (Duplex)

Follicular Lymphoma (1)

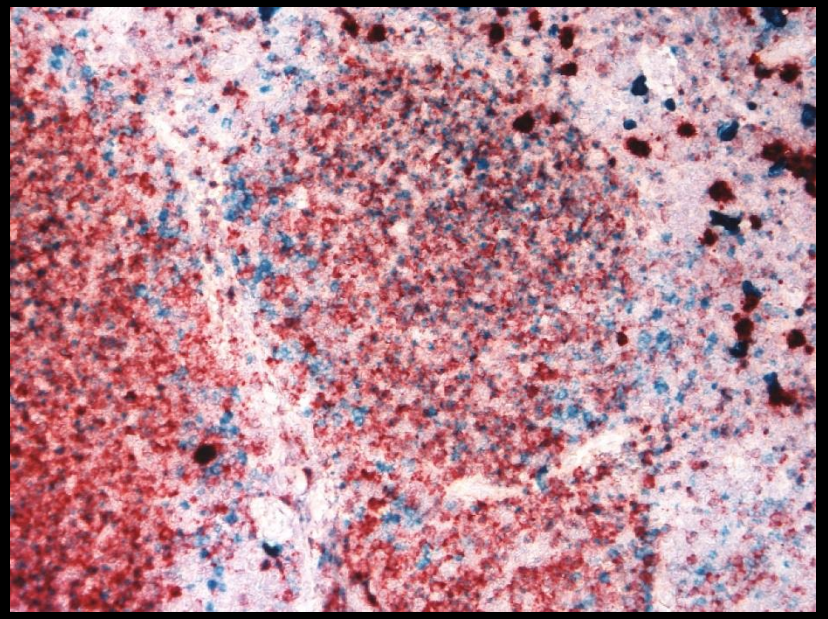
Clinical info: Kappa positive (Ros)



IHC Lambda (re-test)



IHC Kappa (re-test)



RNA scope Kappa-(C2-Red) Lambda-(C1-Green)

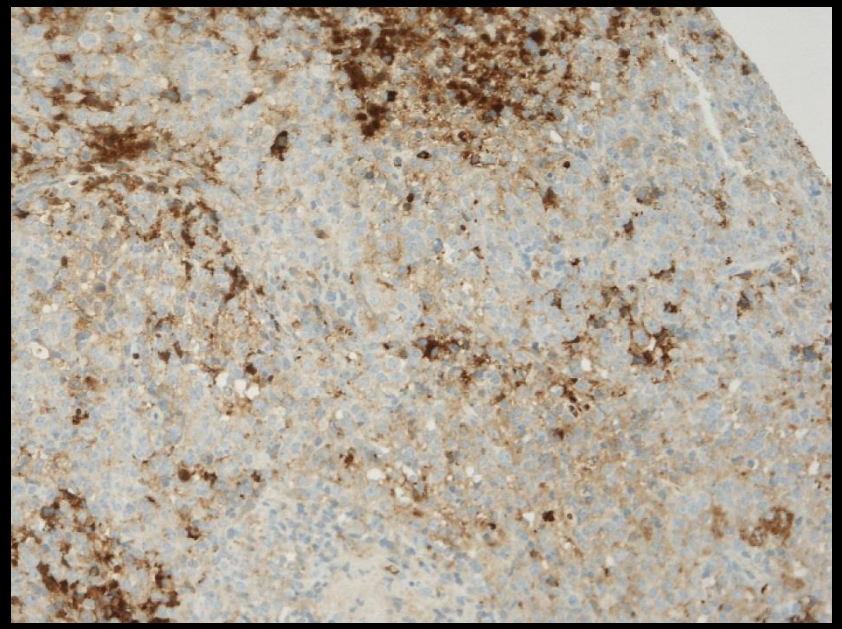
Significant proportion of IGLL5 (n) + cells

H15/P10-20-30

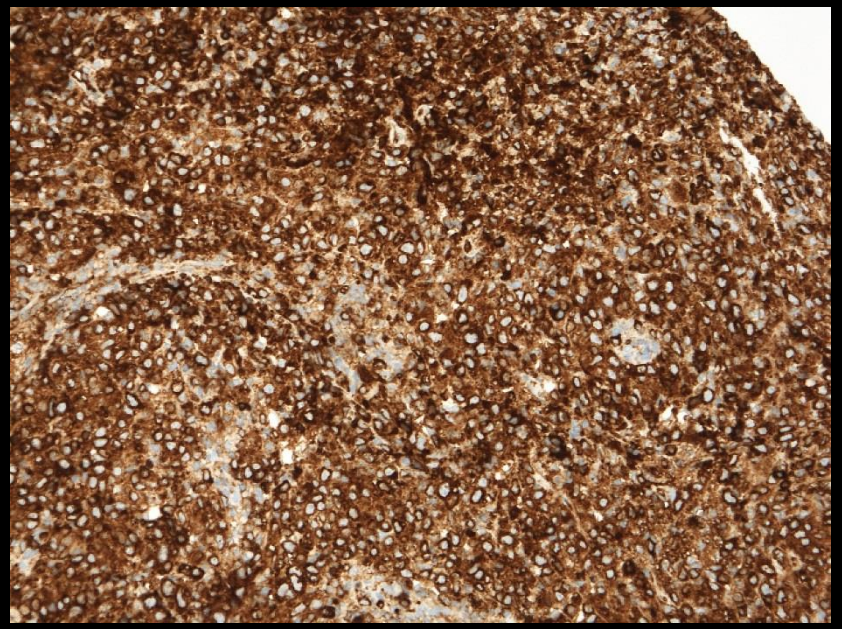
RNA Scope

Diffuse Large B-Cell Lymphoma (1)

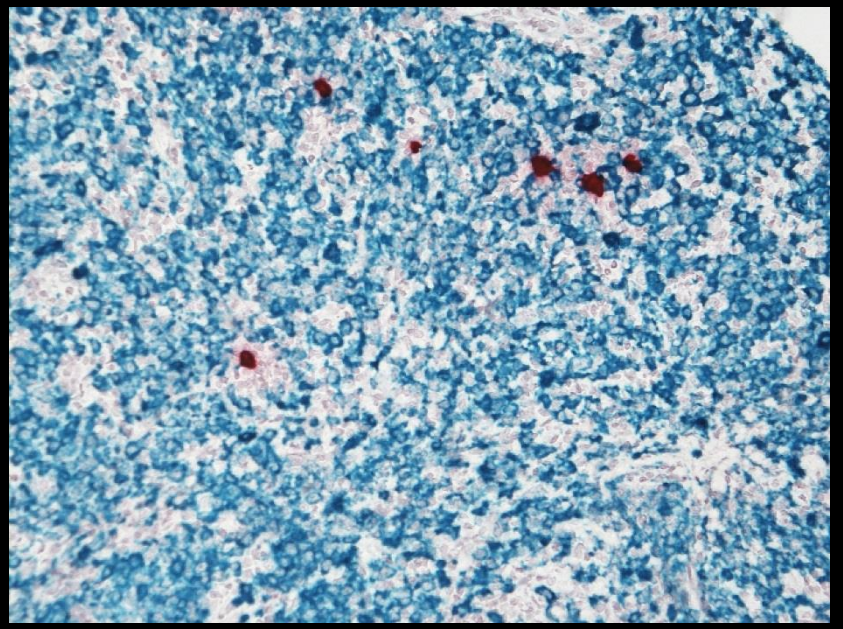
Clinical info: Lambda positive (Ros)



IHC Kappa (re-test)



IHC Lambda (re-test)



RNA scope Kappa (C2-Red)/Lambda (C1-Green)

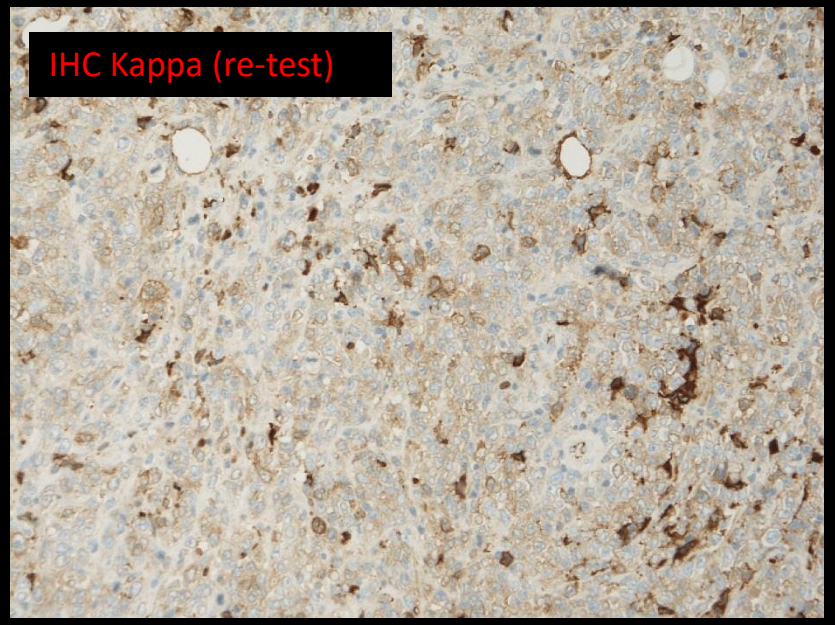
H15/P10-20-30

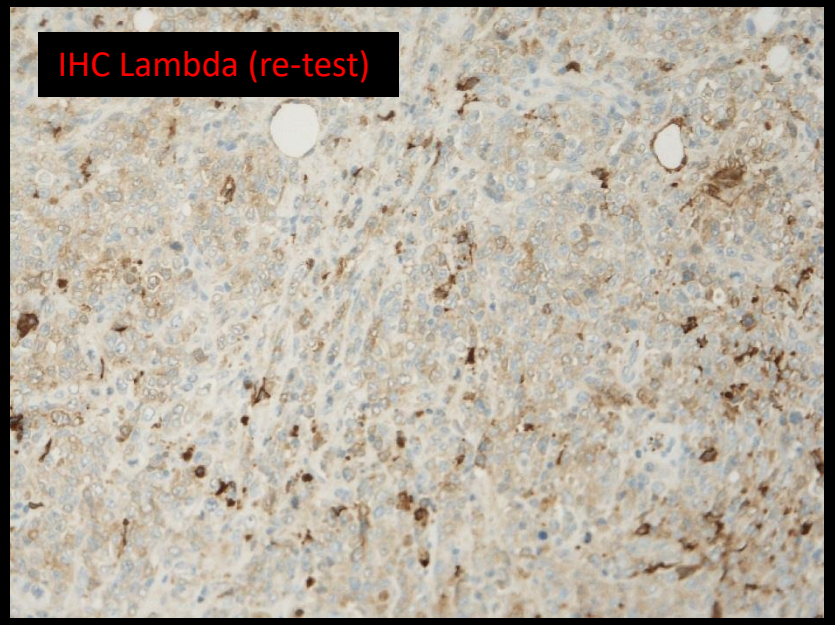
RNA Scope

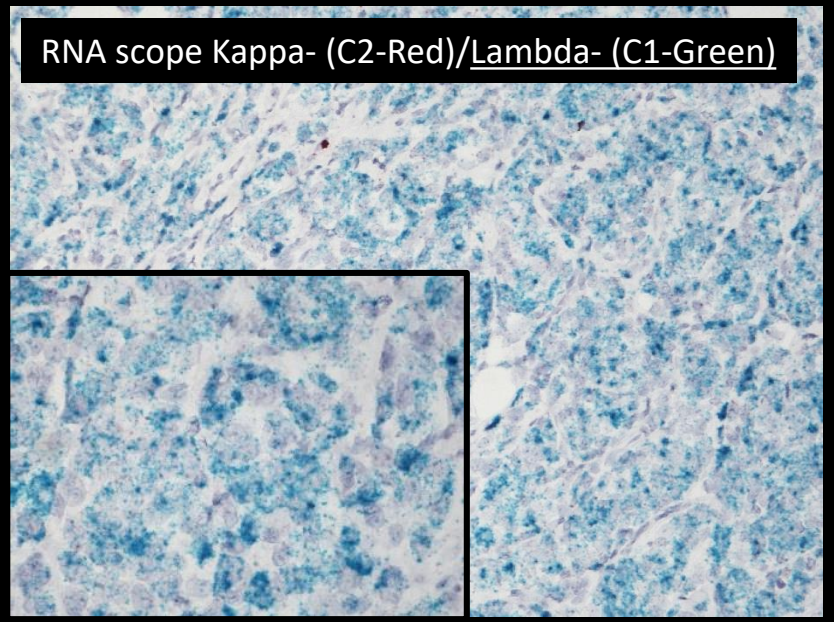
Diffuse Large B-Cell Lymphoma (2)

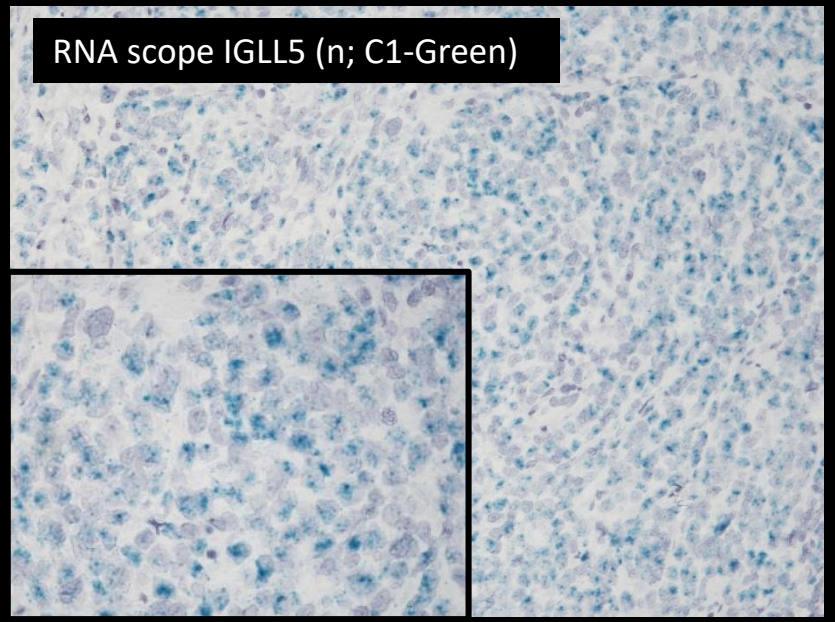
Clinical info: Unknown

Difficult case (Lambda + ?)







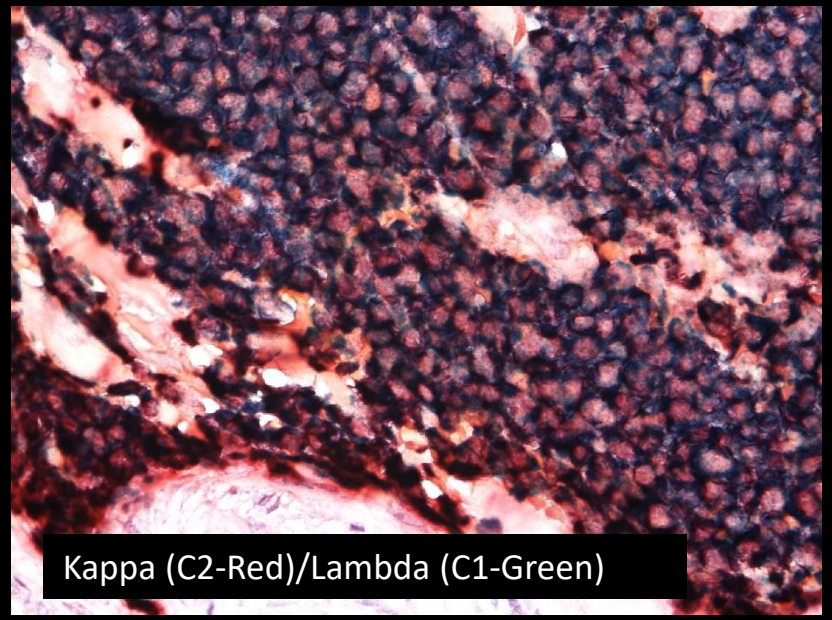


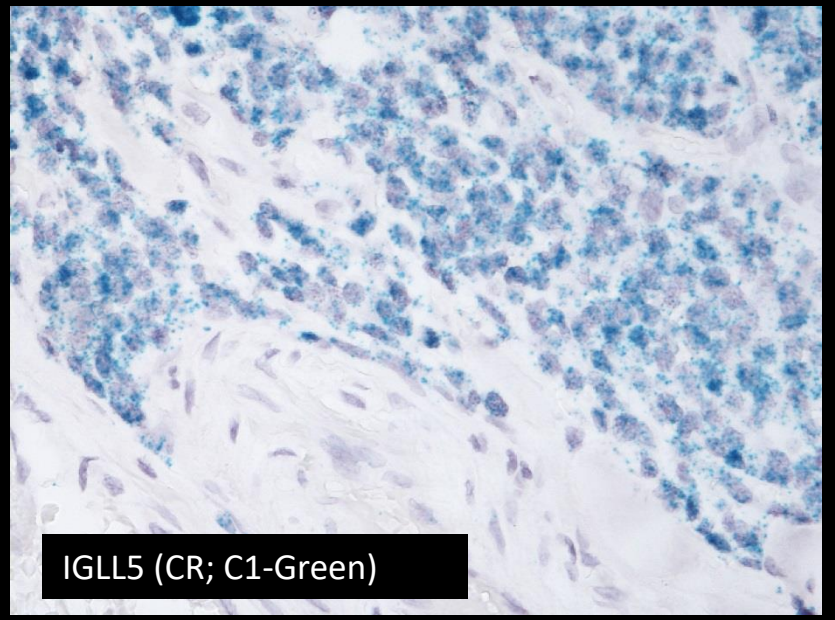
H15/P15

The problems !

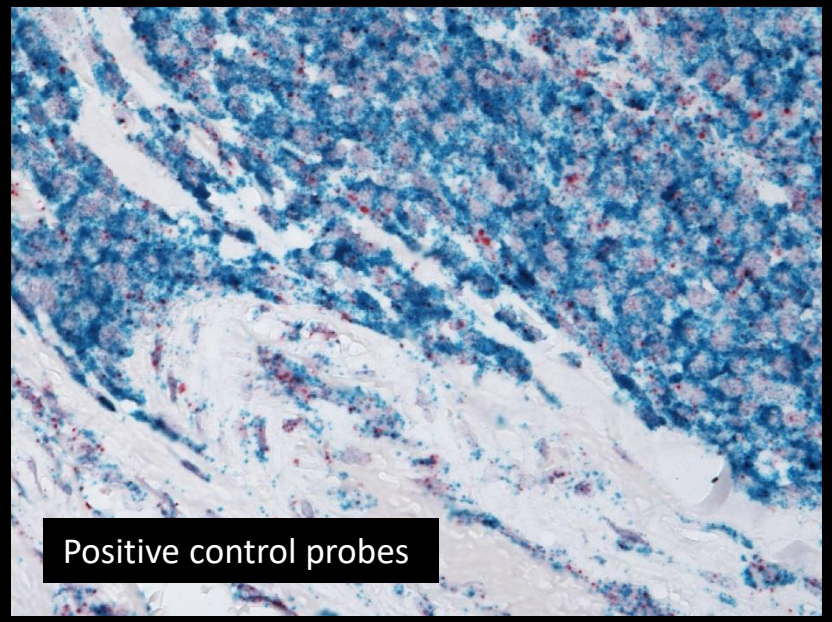
RNA Scope Duplex

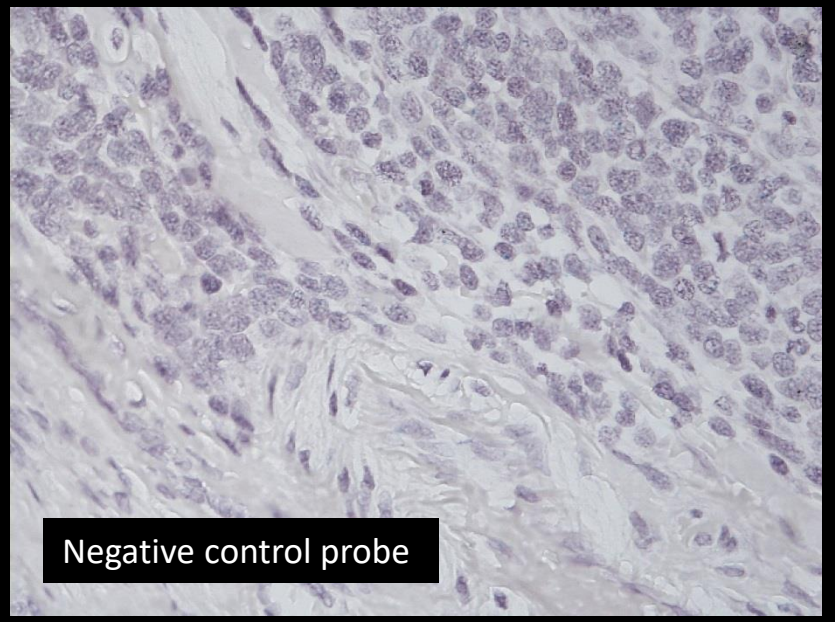
Myeloma (Kappa +)





“Cross-reactivity” IGLL5+





H15/P10-20-30

RNA Scope Duplex

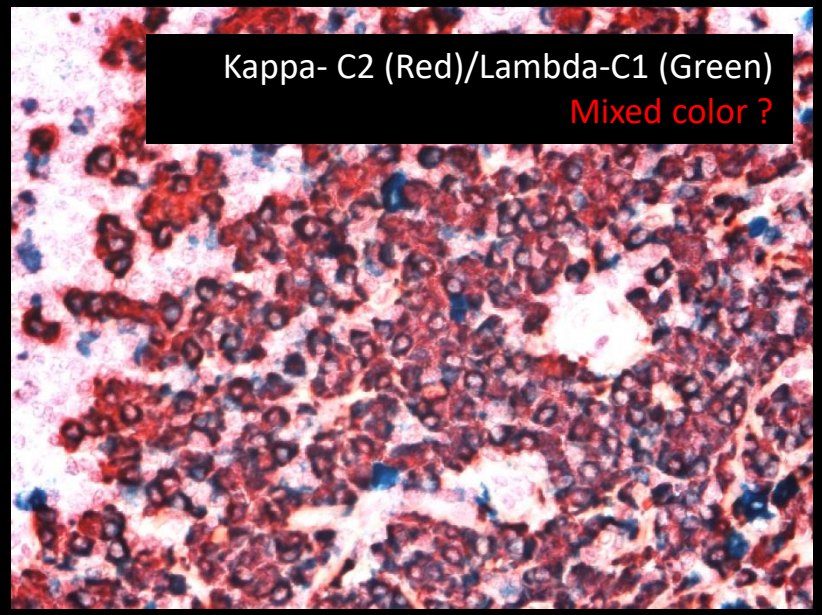
With/Without Lambda probe

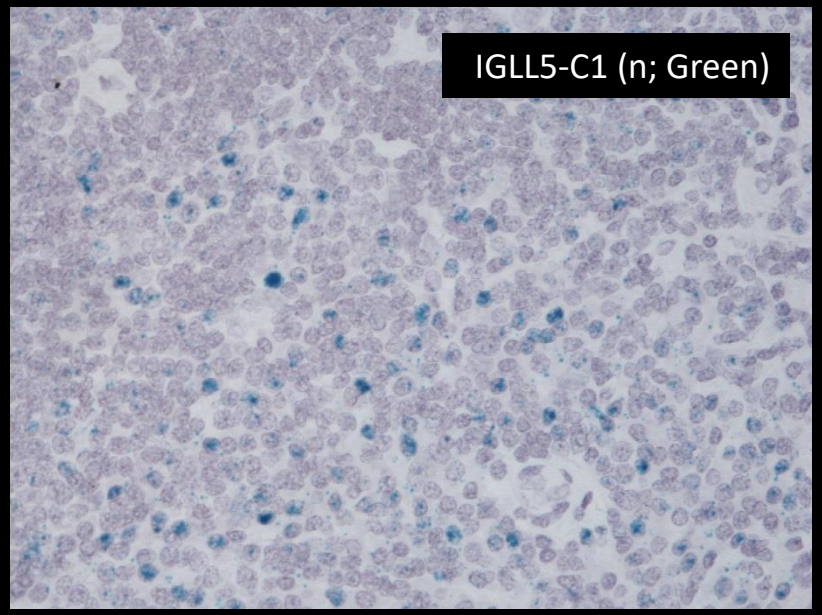
LPL (case 3)/ Kappa +

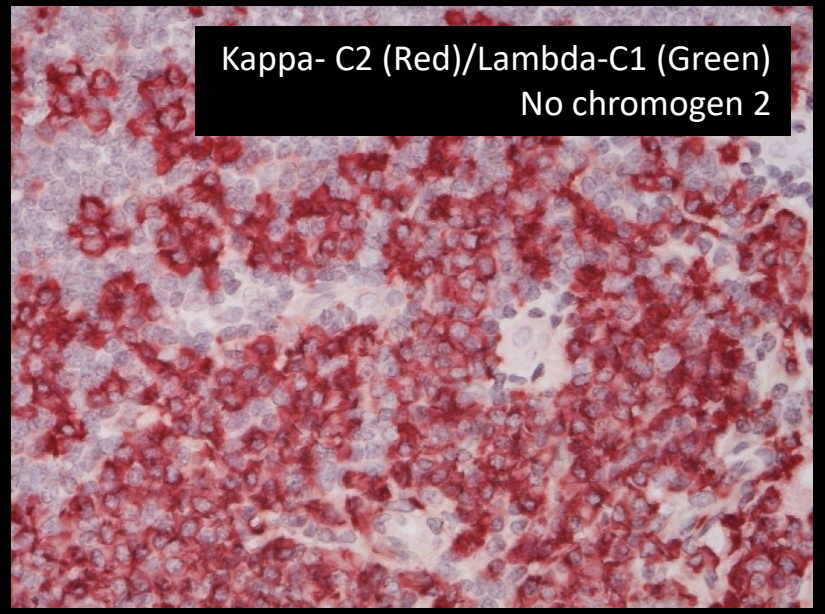
Cross-reactivity with Lambda probe ?

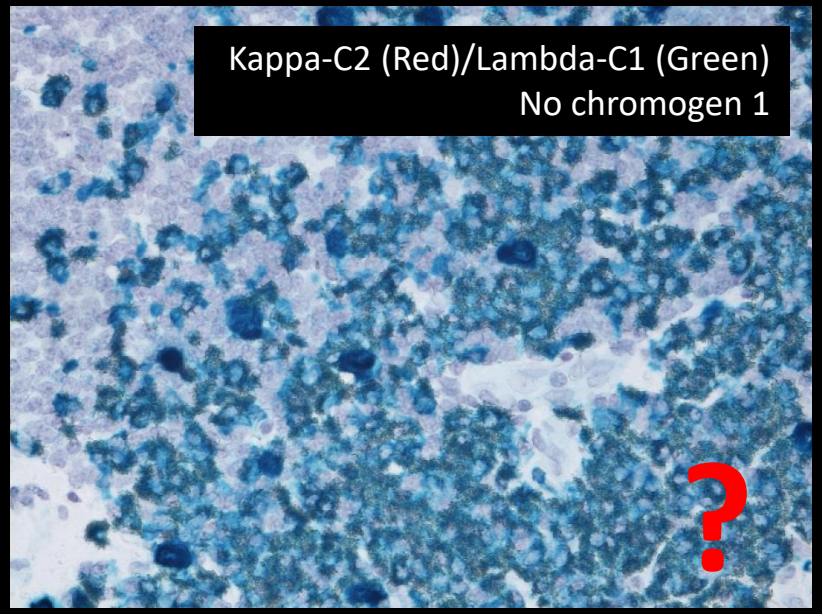
Detection system – cross talk ?

No reaction were seen with Kappa/Lambda/IGLL5 probe in non-lymphoid tissue e.g. trophoblastic cells of the placenta, epithelial/stromal cells of the all specimens. Positive and negative controls displayed the expected reaction pattern in all specimens.





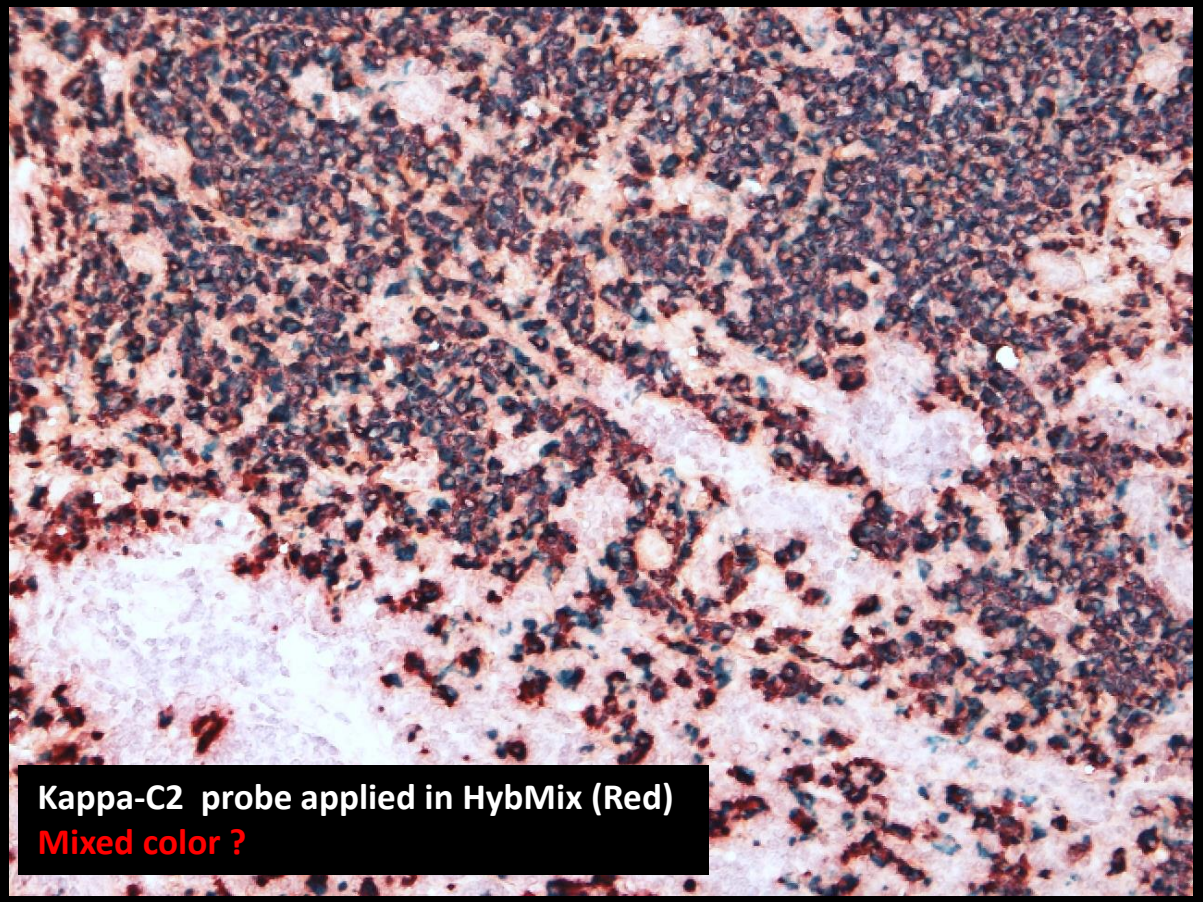


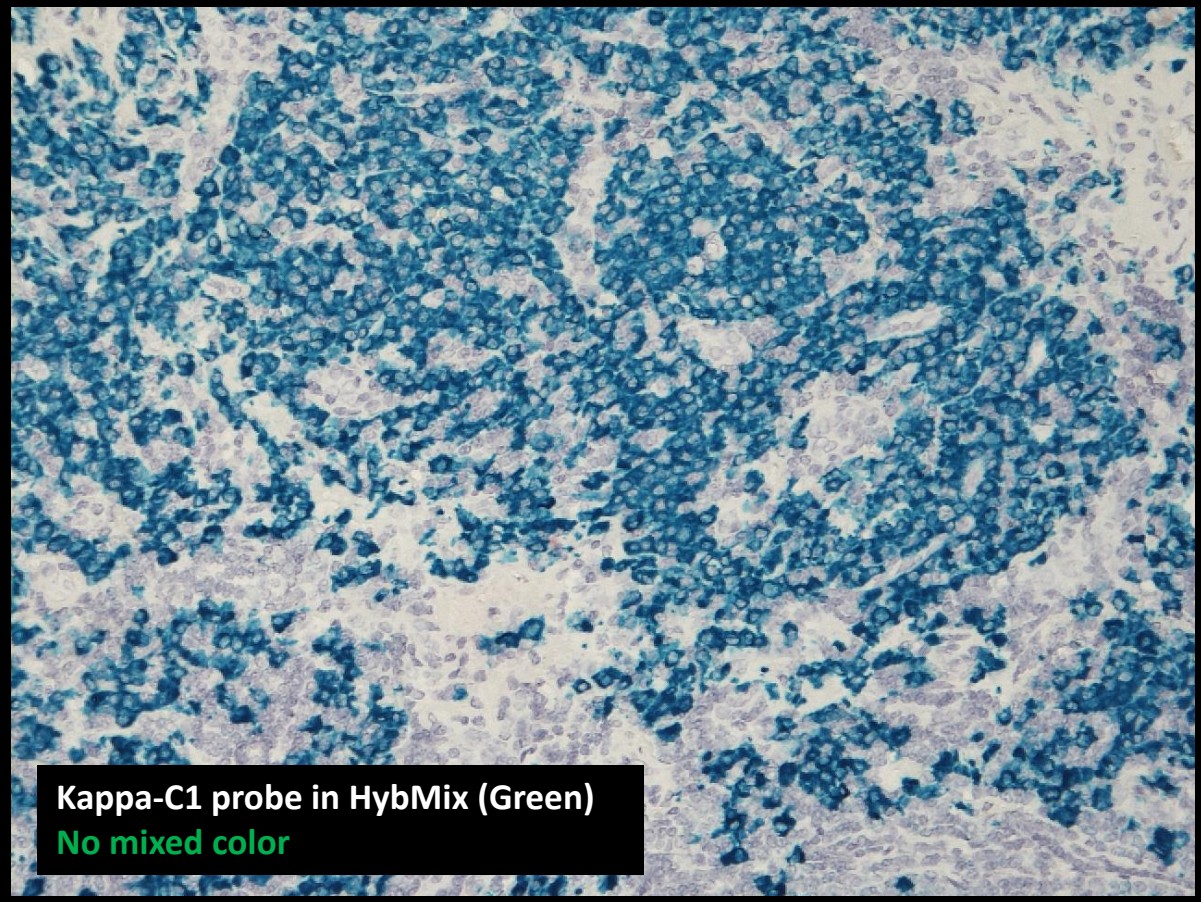


H15/P10-20-30

RNA Scope Duplex: Kappa-C2 versus Kappa-C1

LPL (case 3)/ Kappa +





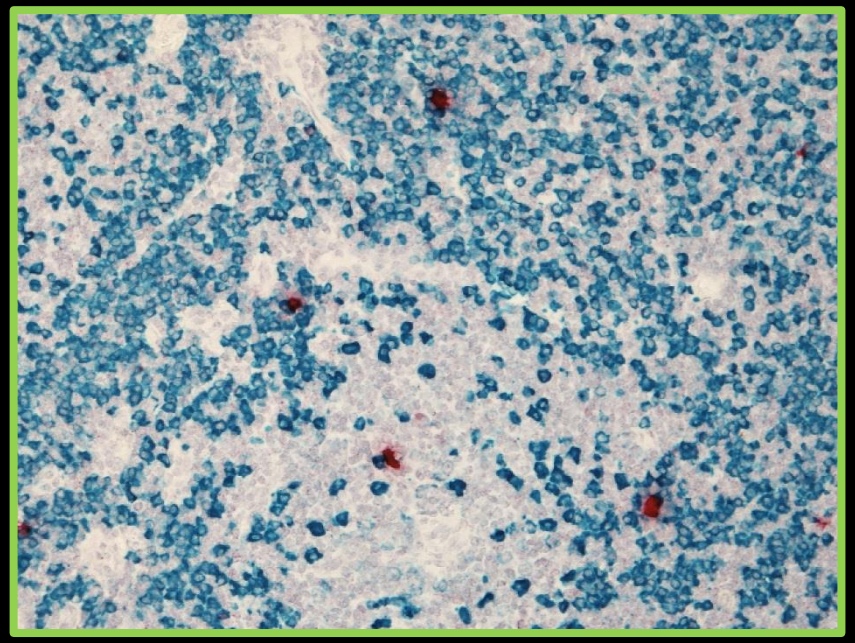
Problems related to abundant expression of a given target mRNA type, e.g, Kappa positive LPL cases, and application of corresponding C2 probe to the same target.

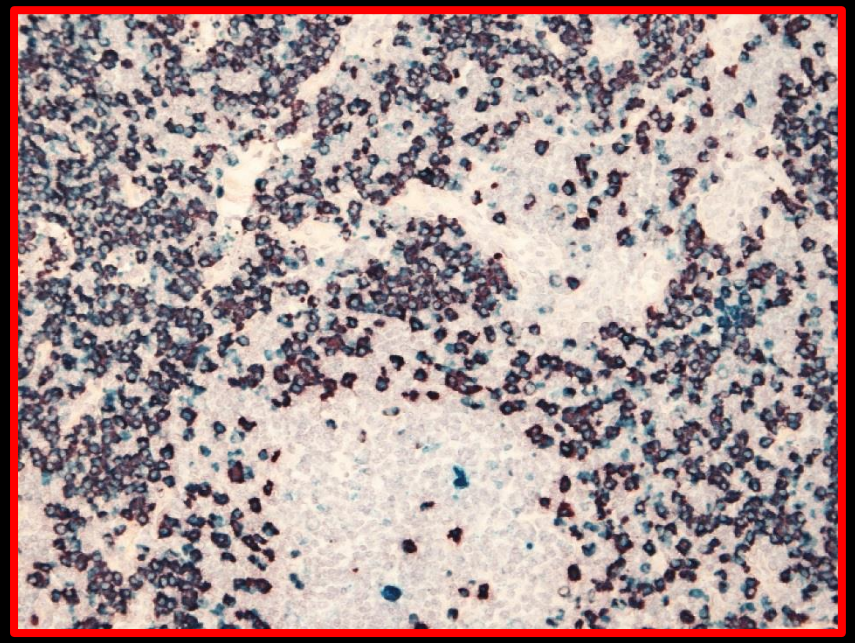
RNA Scope Duplex

IGL (C1) + Kappa (C2)

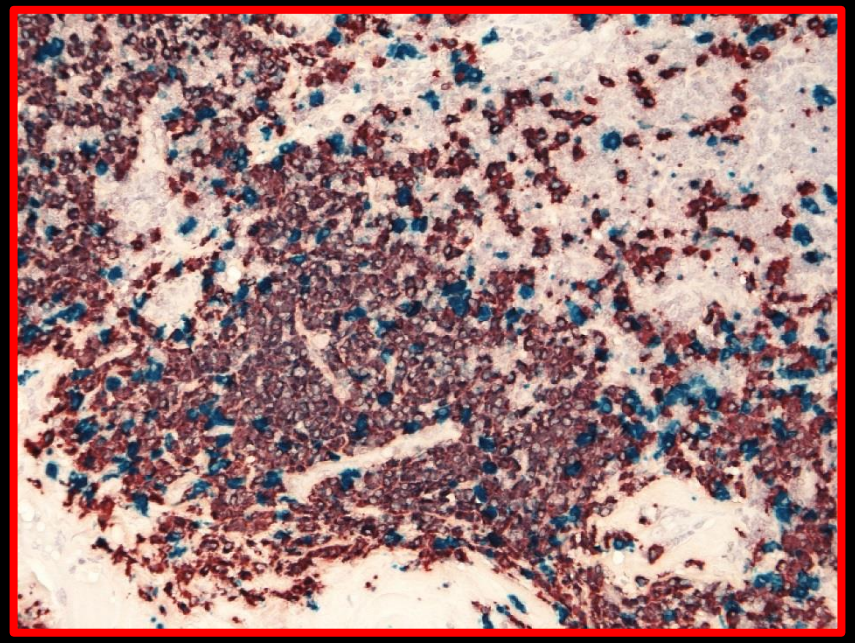
IGL (C2) + Kappa (C1)

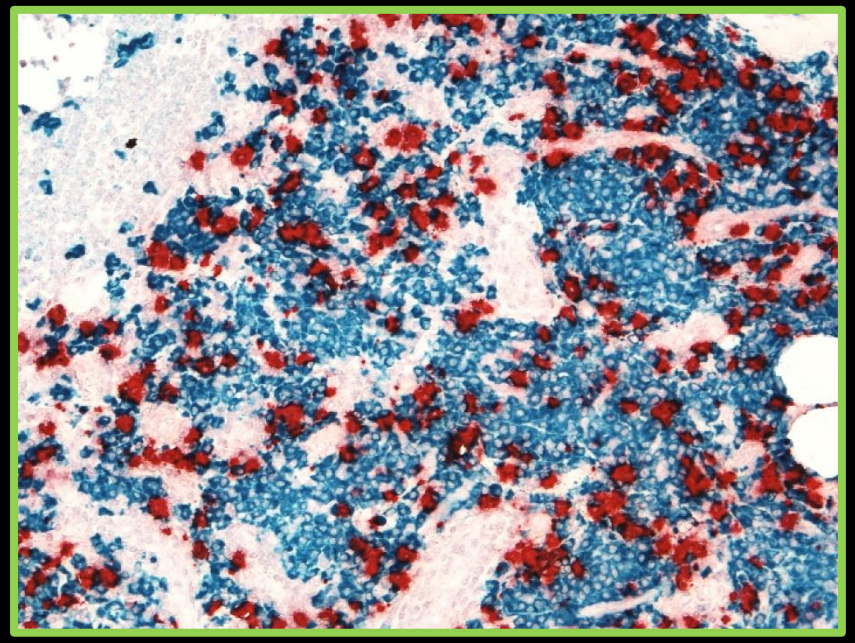
LPL (Lambda +)





LPL (Kappa +)





Advance Cell Diagnostic (RNAscope) respond:

This means the problem is with high C2 signal that creates unspecific green signal overlapping with the red, which follows the expected pattern of the C2 (red) target.

And it turns out that this is actually something we expect for the RNAscope and BaseScope duplex assays. We always recommend to put the highest expressor in C1, because we know that a lot of red signal can interfere with the green signal.

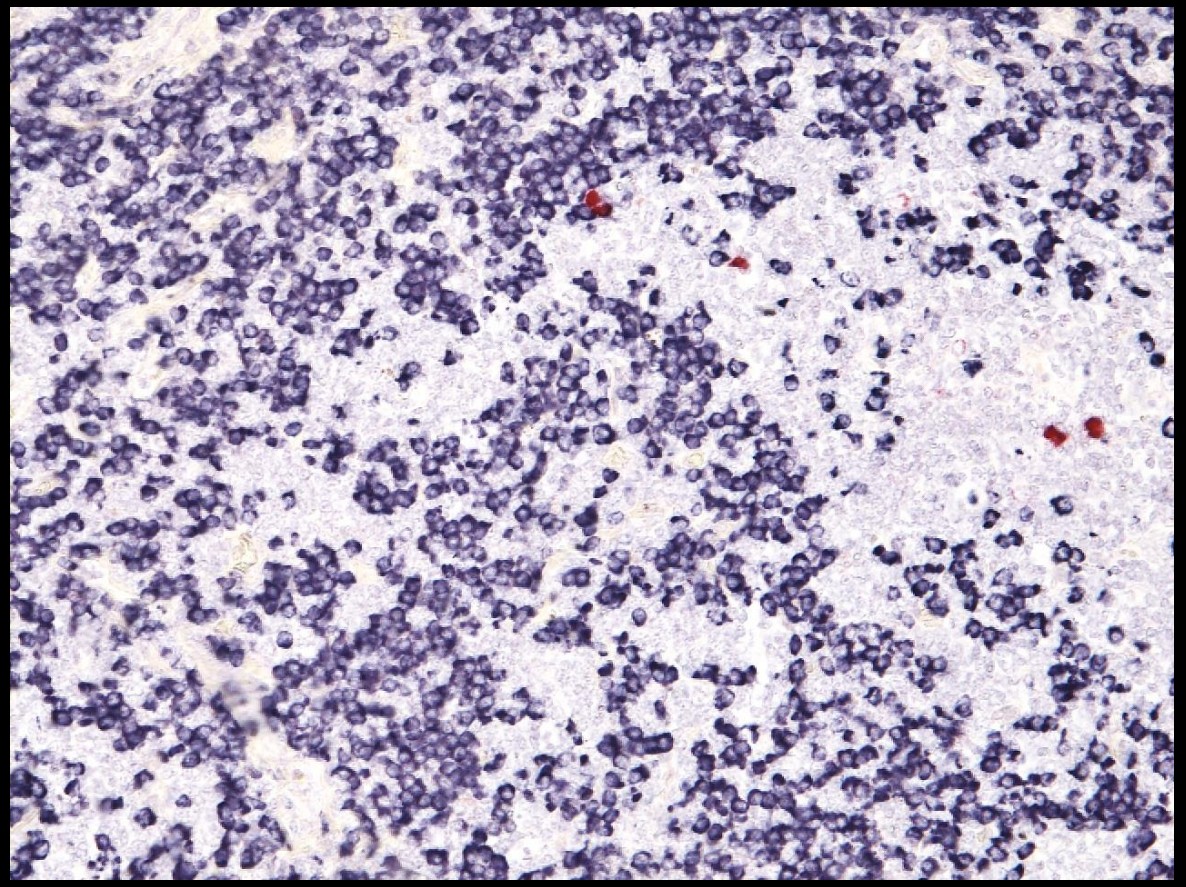
However, we rarely see any problem even if customers pick C2 for a target that is a bit higher than that in C1 and we understand that it is not always possible to know this in advance.
But, Kappa and Lambda tissues are the “extreme” of this situation, where Kappa or Lambda can be very very high. And it is exactly for cases like this that we have this rule.

So, fundamentally there is nothing wrong, but we are dealing simply with a limit of the RNAscope duplex chemistry and there is no way around it if not switching the probes for samples where you see this happening.

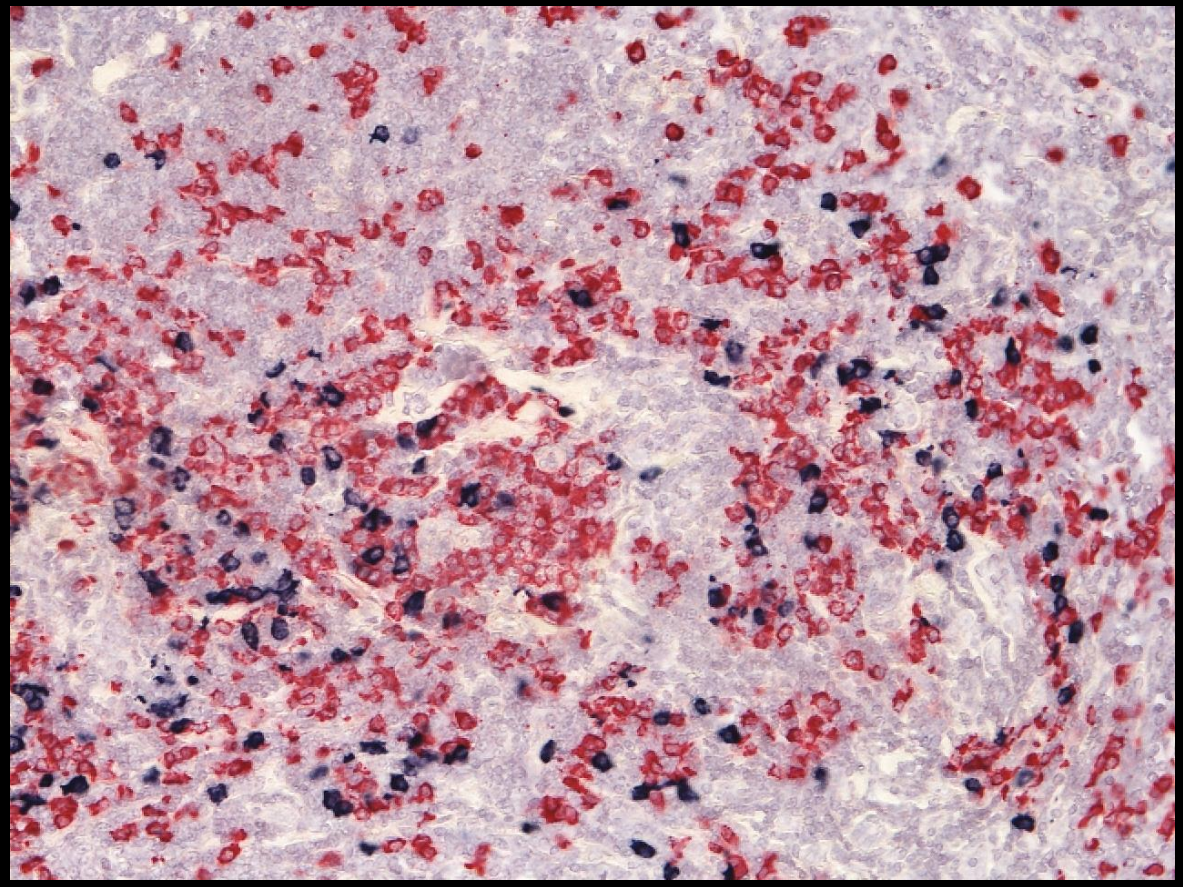
Trouble shooting guide ?

ViewRNA 2-Plex: Lambda (Type 6 probe/Blue) and Kappa (Type 1 probe/Red)

LPL (Lambda positive)



LPL (Kappa positive)



Preliminary result for the ViewRNA: Assay needs optimization

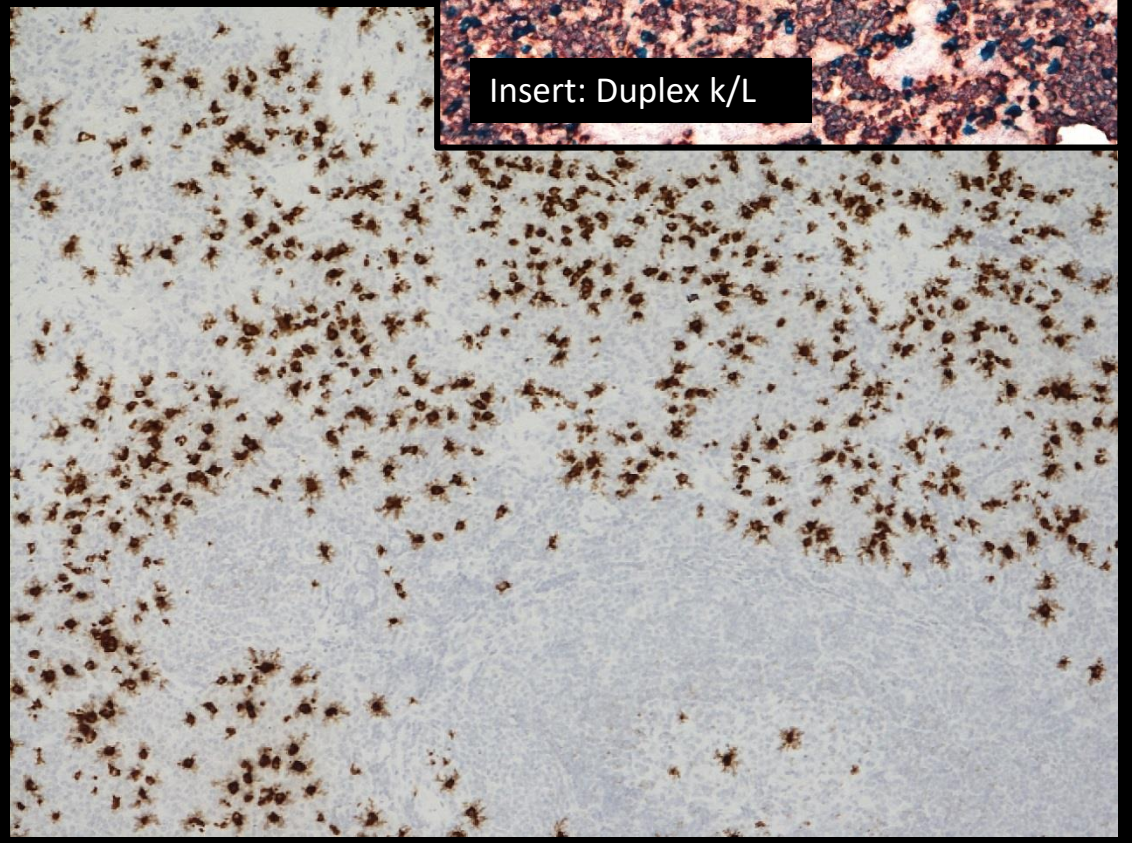
No cross talk

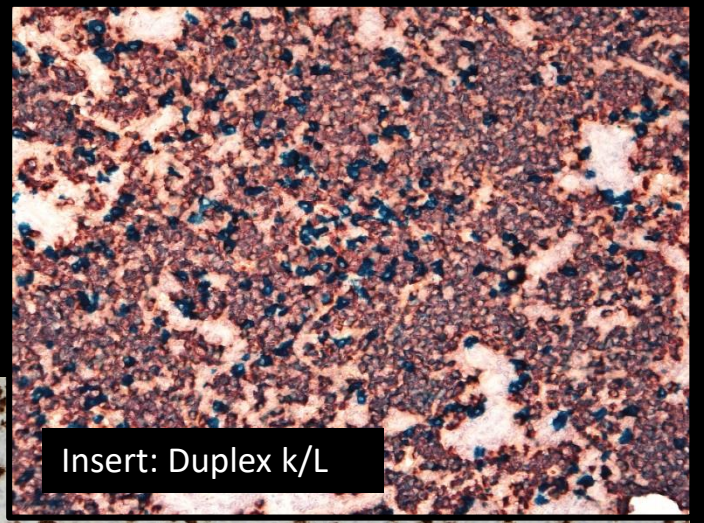
RNA Scope (Single Plex)

LPL (case 3)

Kappa +

Kappa-C1 (Single Plex)





Lambda-C1 (Single Plex)

No cross-reactivity

Diagnosis	Clinical info Light chain restriction	RNA Scope : C1 probe Lambda/ C2 probe Kappa Light chain restriction
Lymphoplasmacytoid lymphoma (LPL) (1) Næ	Lambda$^+$/Kappa$^-$ (IHC$^+$/ISH$^+$)	Lambda$^+$/Kappa$^-$
Lymphoplasmacytoid lymphoma (LPL) (2)/Næ	Lambda$^+$/Kappa$^-$ (IHC$^+$/ISH$^+$)	Lambda$^+$/Kappa$^-$
Lymphoplasmacytoid lymphoma (LPL) (3)/Næ	Kappa$^+$/Lambda$^-$ (IHC$^+$/ISH$^+$)	Kappa$^+$/Lambda$^-$ (Cross-reactivity ?)
Lymphoplasmacytoid lymphoma (LPL) (4)/Ros	Kappa$^+$/Lambda$^-$	Kappa$^+$/Lambda$^-$ (Cross-reactivity ?)
Lymphoplasmacytoid lymphoma (LPL) (5)/Ros	Kappa$^+$/Lambda$^-$	Kappa$^+$/Lambda$^-$ (Cross-reactivity ?)
Lymphoplasmacytoid lymphoma (LPL) (6)/Ros	Kappa$^+$/Lambda$^-$	Kappa$^+$/Lambda$^-$ (Cross-reactivity ?)
Lymphoplasmacytoid lymphoma (LPL) (7)/Ros	Unknown (re-test displayed Kappa IHC$^+$/ISH$^+$ result)	Kappa$^+$/Lambda$^-$ (Cross-reactivity ?)
Lymphoplasmacytoid lymphoma (LPL) (8)/Ros	Unknown (re-test displayed Kappa IHC$^+$/ISH$^+$ result)	Kappa$^+$/Lambda$^-$ (Cross-reactivity ?)
Myeloma/Næ	Kappa$^+$/Lambda$^-$ (IHC$^+$/ISH$^+$)	Kappa$^+$/Lambda$^-$ (difficult IGLL5 reaction)
Mantle cell lymphoma (MCL) (1)/Næ	Kappa$^+$/Lambda$^-$ (IHC$^+$/ISH$^-$)	Kappa$^+$/Lambda$^-$
Mantle cell lymphoma (MCL) (2)/Næ	Kappa$^+$/Lambda$^-$ (IHC$^+$/ISH$^-$)	Kappa$^+$/Lambda$^-$
Mantle cell lymphoma (MCL) (3)/Næ	Lambda$^+$/Kappa$^-$ (IHC$^+$/ISH$^-$)	Lambda$^+$/Kappa$^-$
Mantle cell lymphoma (MCL) (4)/Næ	Lambda$^+$/Kappa$^-$ (IHC$^+$/ISH$^-$)	Lambda$^+$/Kappa$^-$
Follicular Lymphoma (FL) (1)/Ros	Kappa$^+$/Lambda$^-$	Kappa$^+$/Lambda$^-$ (difficult IGLL5 reaction)
Follicular Lymphoma (FL) (2)/Ros	Unknown (re-test displayed Lambda IHC$^+$/ISH$^-$ result)	Lambda$^+$/Kappa$^-$
Follicular Lymphoma (FL) (3)/Ros	Kappa$^+$/Lambda$^-$	Interpretation difficult (pre-analytic problems/IGLL5)
Diffuse Large B-Cell Lymphoma (DLBCL) (1)/Ros	Lambda$^+$/Kappa$^-$	Lambda$^+$/Kappa$^-$
Diffuse Large B-Cell Lymphoma (DLBCL) (2)/Ros	Unknown (re-test displayed IHC$^-$/ISH$^-$ result)	Lambda$^+$/Kappa$^-$
Tonsil (Fix time 6-168h)	Poly	Poly/ Germinal centre B-cells (strong IGGL5)
Negative control tissue (Appendix, Kidney and placenta)	Negative	Negative

In general, there is a good correlation between RNA scope results and In House test (Standard ISH, IHC and Flowcytometry). However, ……..

Our approach to mRNA ISH (RNAscope)

- Helpful in challenging diagnostic situations e.g., detection of light chain restrictions (kappa/lambda) in B-cell Lymphomas
- Confirming mRNA findings (Nanostring profiling) – which cells are positive
- Validation/verification of reaction patterns obtained with research antibodies
- Lack of valid primary antibodies

IL17A+CD3

- BaseScope

e.g. point mutation (BRAF V600E in melanomas or colon adenocarcinomas) or gene fusion products

IL17a (Cytokine)

Associated with several chronic inflammatory diseases including psoriasis, rheumatoid artheritis and multiple sclerosis.

Host defenses against bacterial and fungal infections

Associated with anti-tumor or pro-tumor effects in various cancers.

Produced by:

T helper 17 cells (Th17 cells/$CD4^+$), cytotoxic $CD8^+$ T cells (Tc17 cells), γδ T cells, invariant natural killer T cells (iNKT cells) and lymphoid tissue inducer cells (LTi cells)

Mast cells, neutrophil granulocytes, ……

	Tonsil NBF 24 h. 15-218117	Skin NBF 3 d. 17-500003	Appendix NBF 4 d. 20-20226	Tonsil NBF 24 h. 15-207543
	Tonsil NBF 48 h. 15-218117	Pilonidal Abcess NBF 48 h. 15-7737	Liver NBF 72 h. 16-16101 (OUH)	
	Tonsil NBF 120 h. 15-218117	Placenta NBF Routine 19-208290	Placenta NBF 24 h. 11	
Cell Lines	PSOR2 Psoriasis T-cells IL17A^{-}CD3E^{+} IHC: CD3^{+}	SeaX T-lymphoma IL17A^{+}CD3E^{-} IHC: CD3^{-}	MF2059 Cut. T-Lymph. IL17A^{-} CD3E^{-} IHC: CD3^{-}	

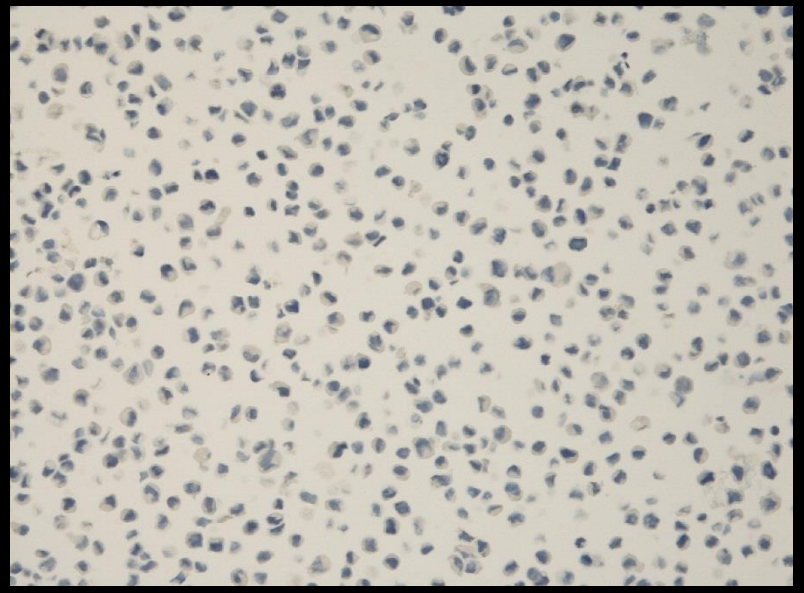
TMA RNA Scope (IL17A)

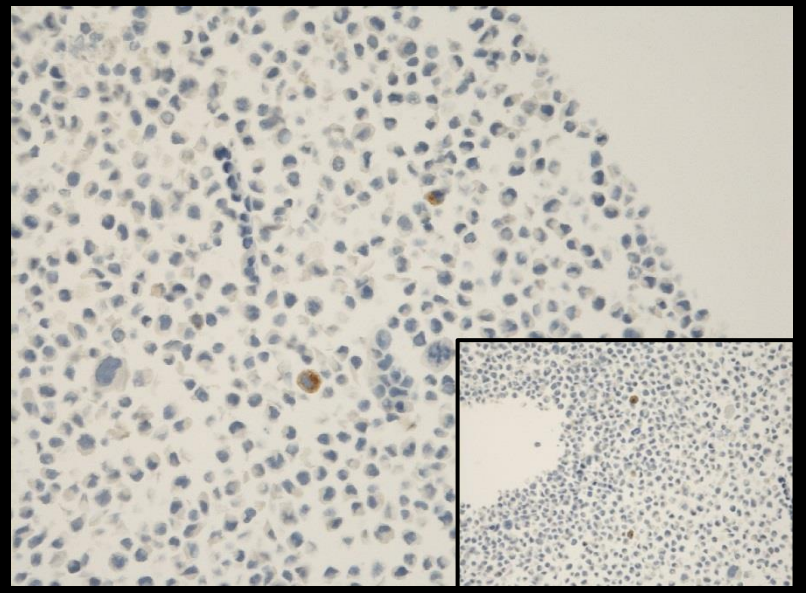
RNAscope (Duplex)

PSOR2 (IL17A-/CD3+)

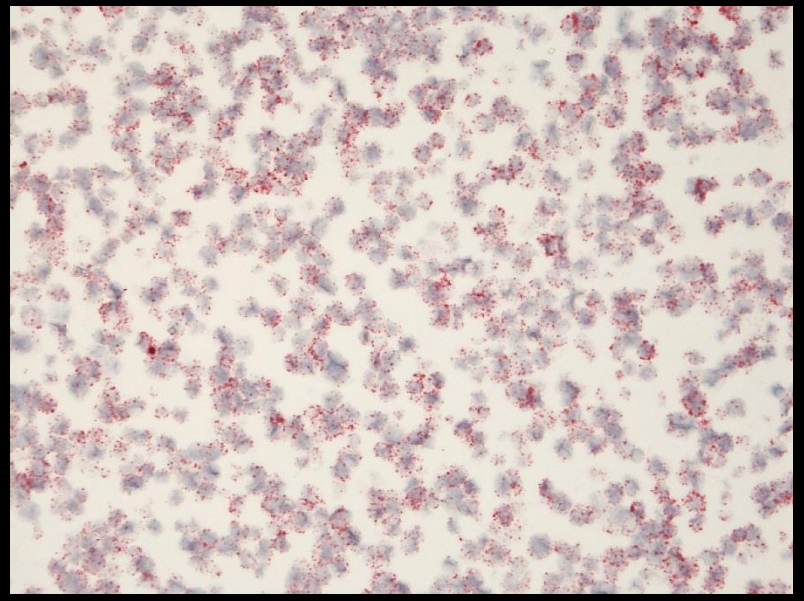
SeAX (IL17A+/CD3-)

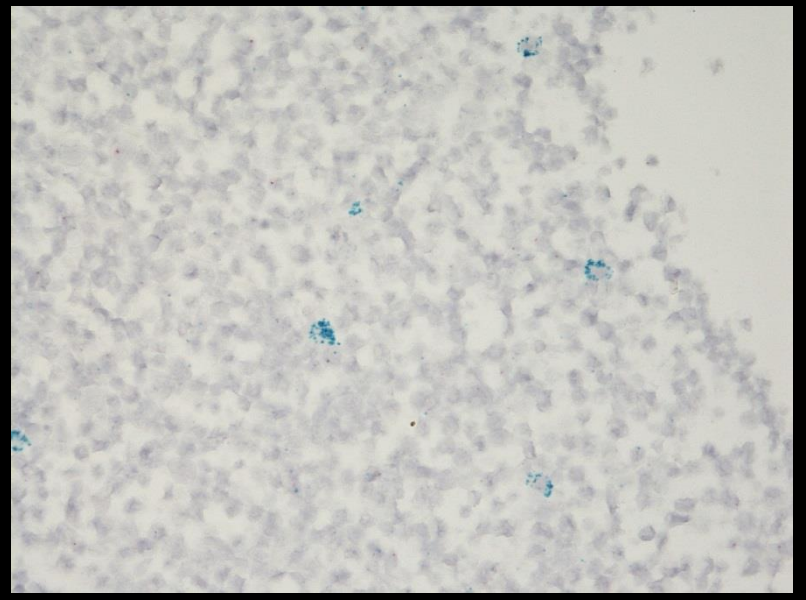
IL17a (Goat polyclonal) IHC





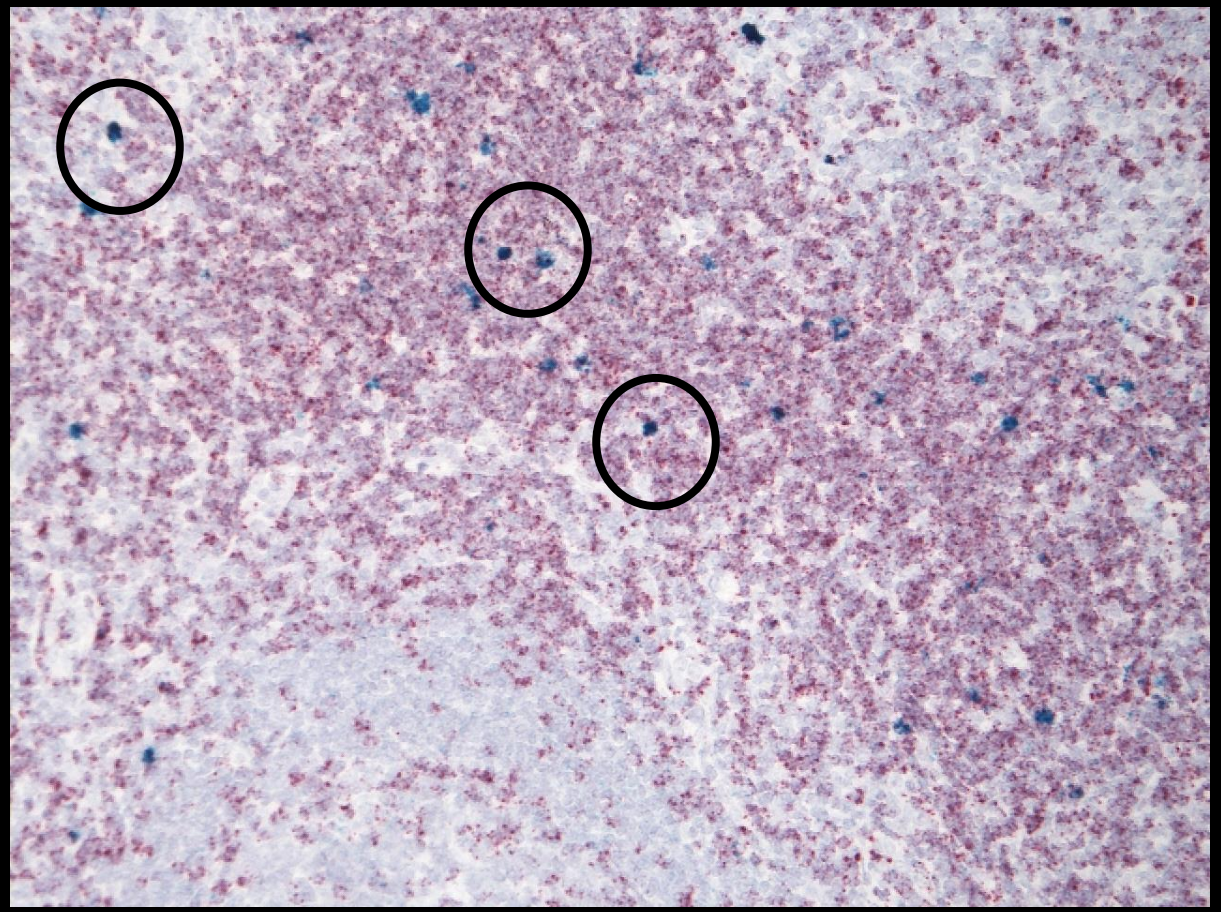
IL17a+CD3E /RNAscope



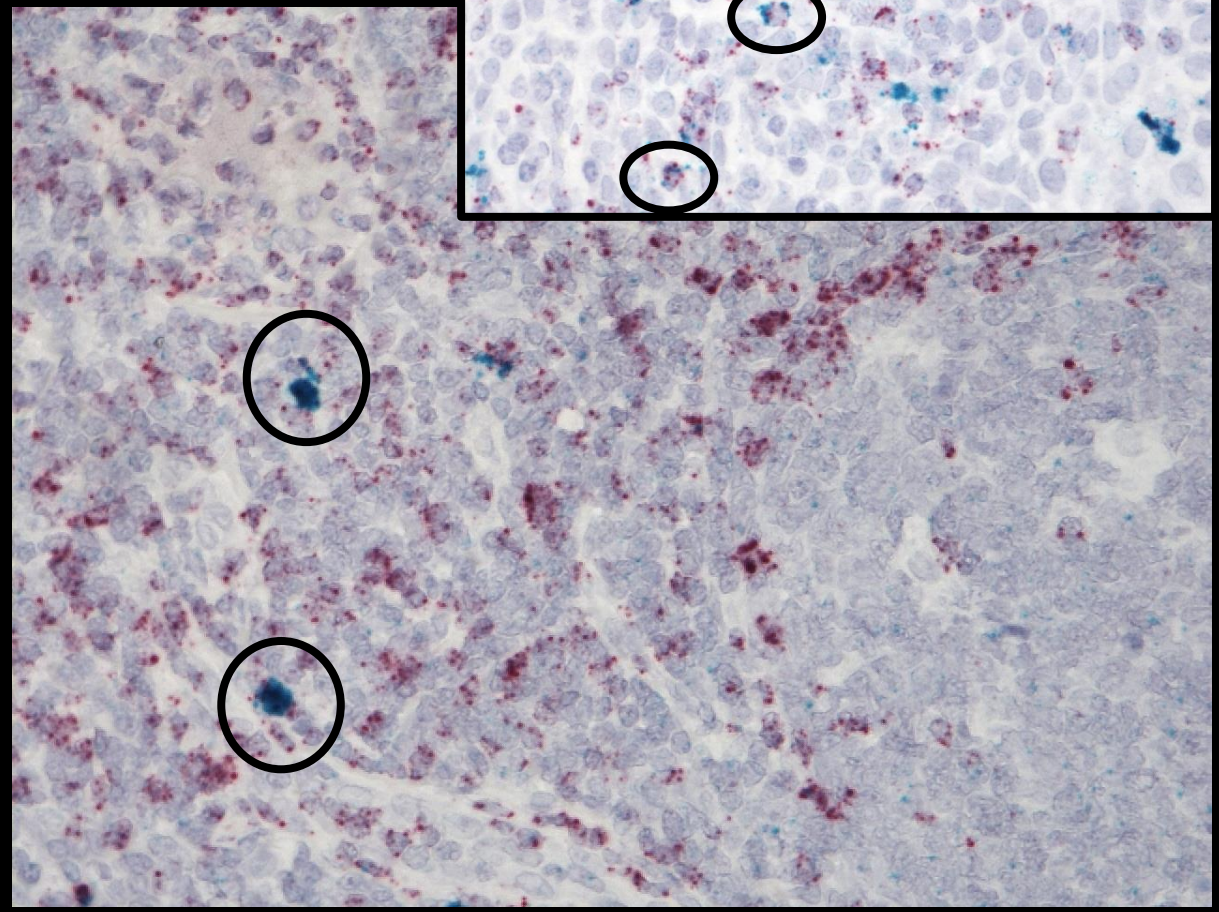


RNAScope Duplex

IL17A: Green/bluish
CD3E: Red
Tonsil



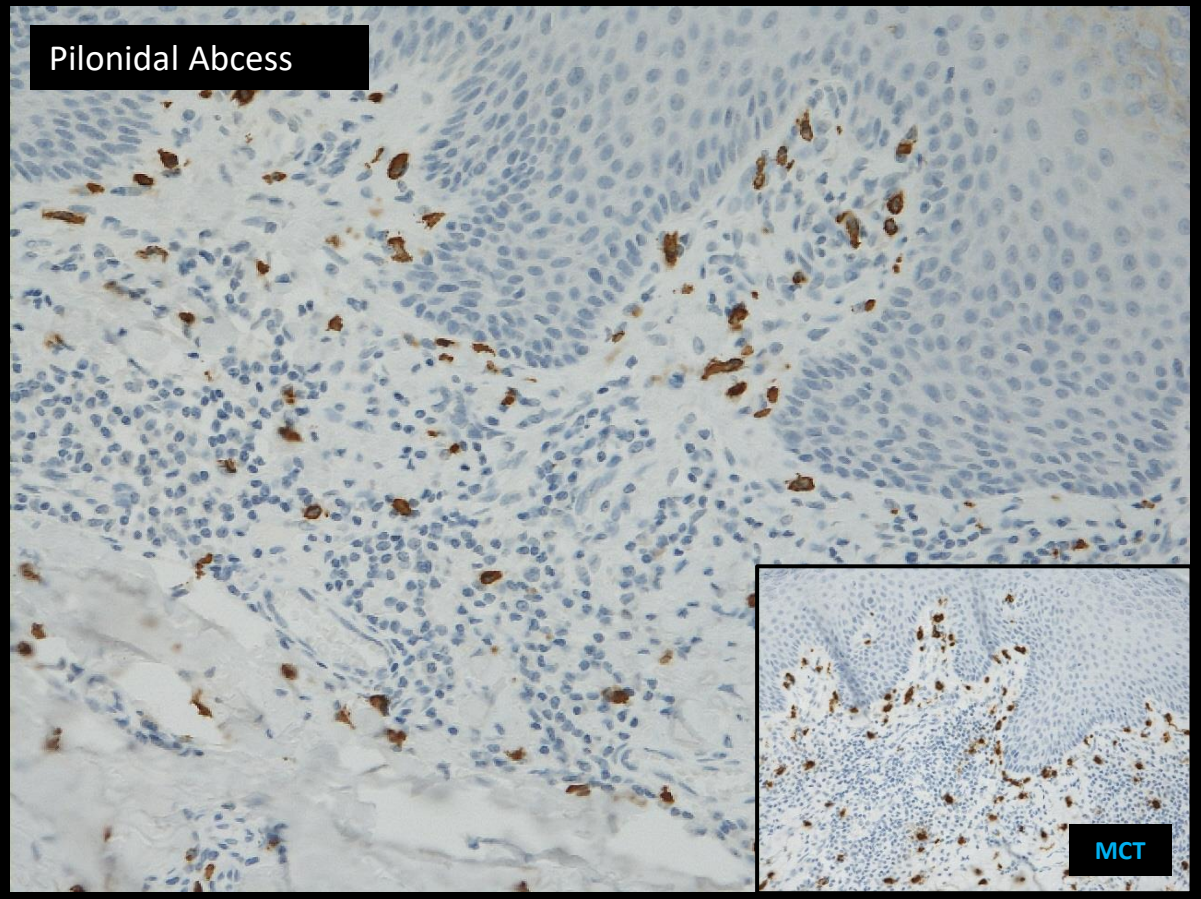
IL17A^{+}CD3E^{+} T-cells / IL17A^{-}CD3E^{+} T-cells



IL17A^{+}CD3E^{+} T-Cell ? Difficult to interpret due to very strong reaction for IL17A. The positive IL17A^{+} are large ?

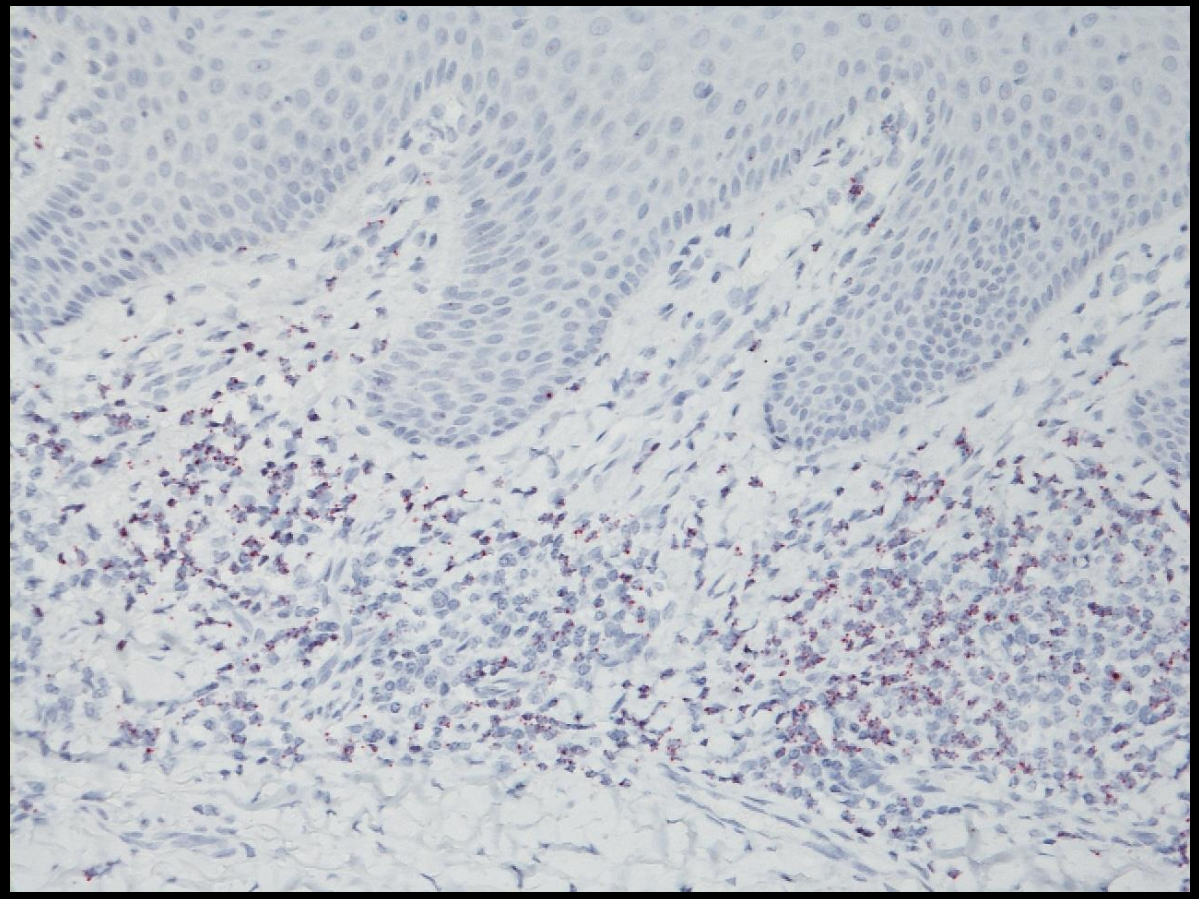
IL17A: The Big Issue ?

Immunohistochemistry: IL17A (polycloal Goat)



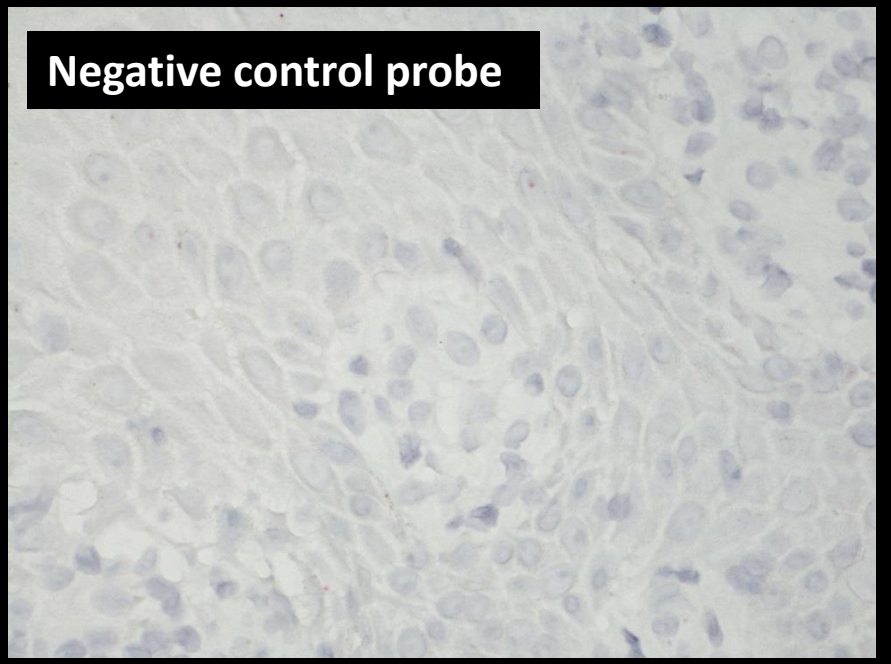
The mast cells/neutrophil granulocytes are positive ?

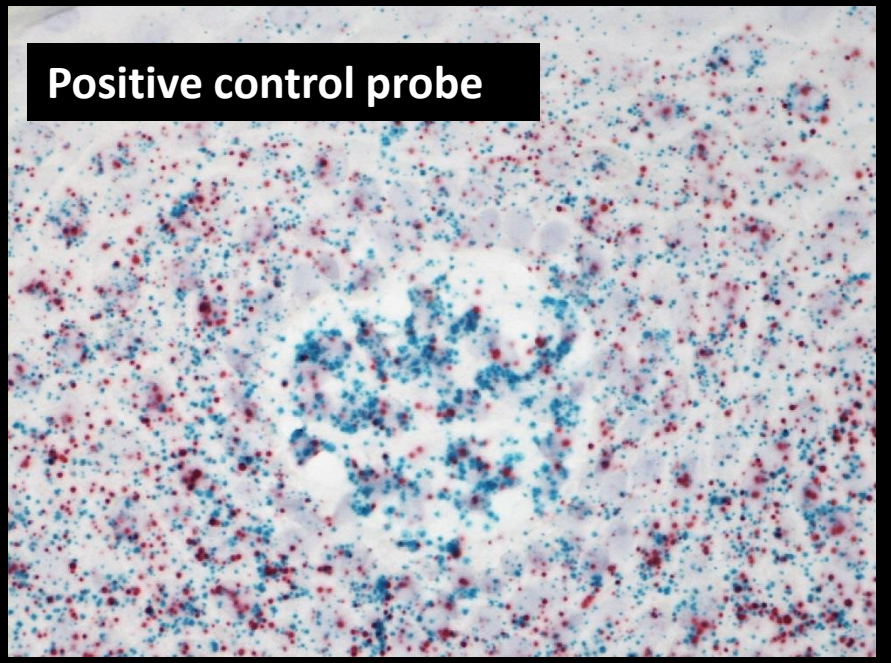
RNAScope Duplex: IL17A+CD3E



Only T-cells are demonstrated (red granular reaction) ?

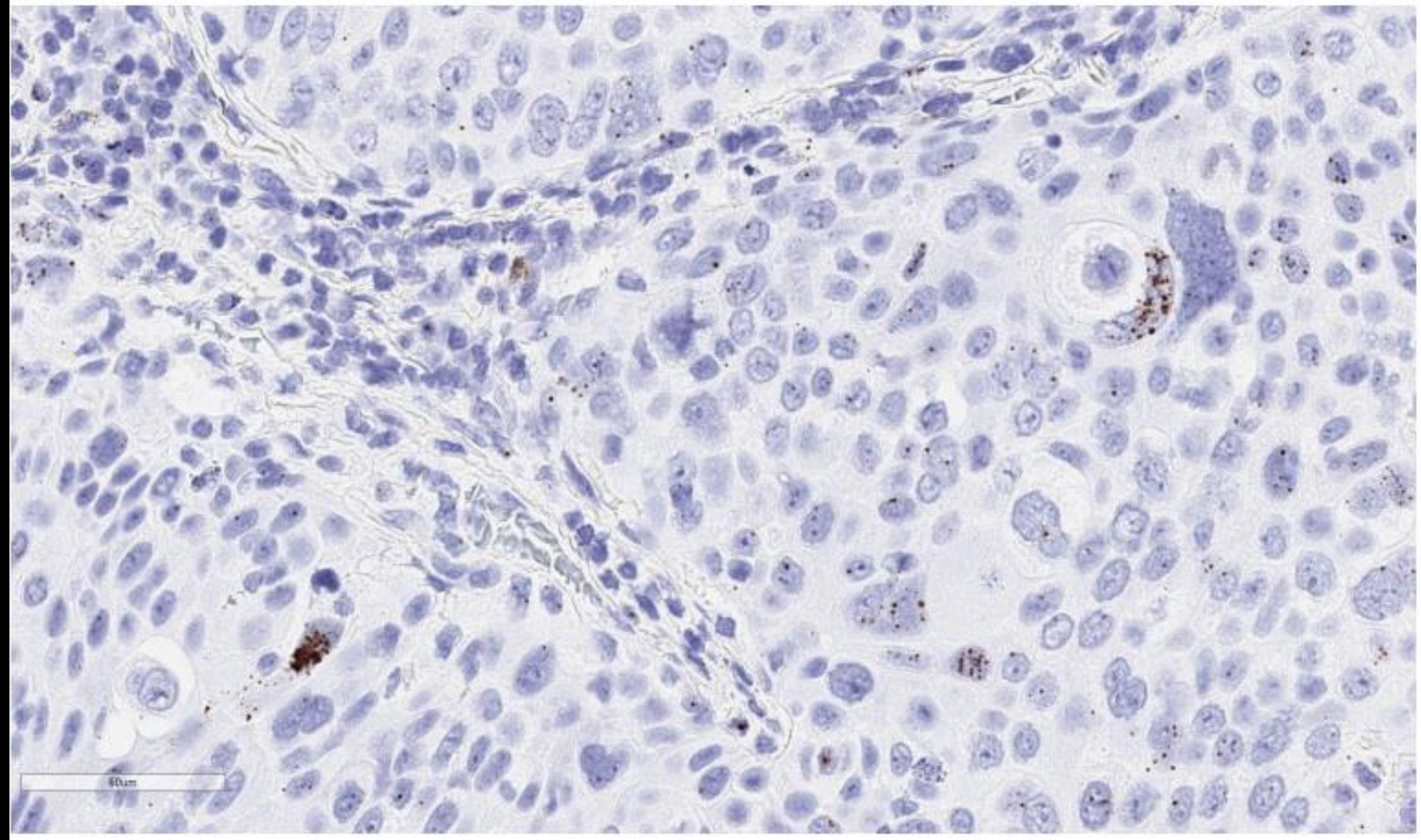
Final thoughts and remarks





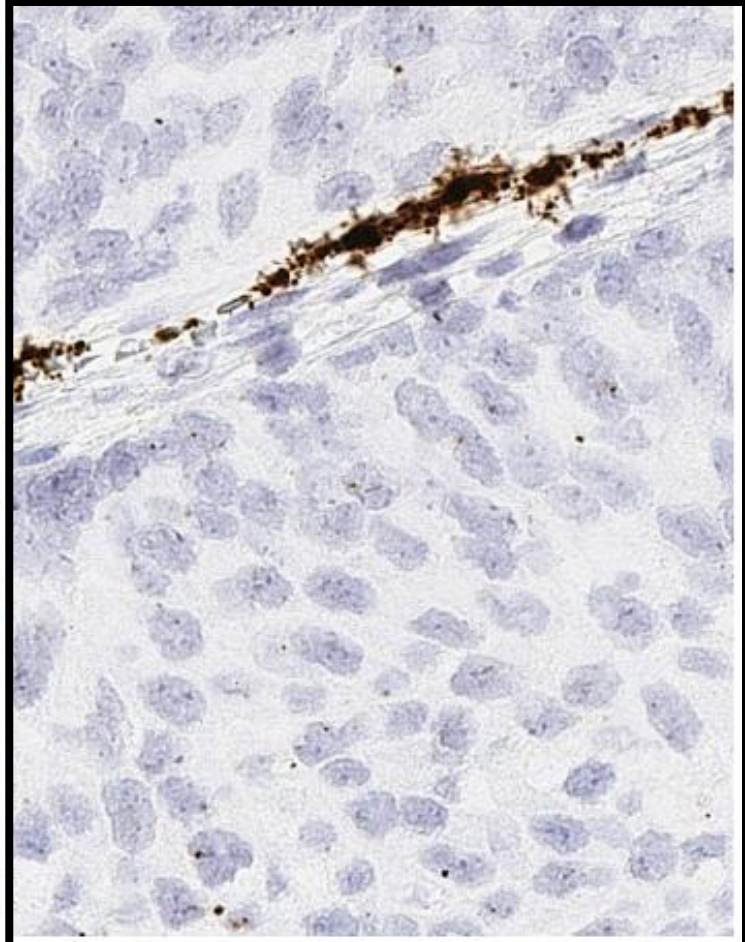
Small dot`s in nuclei`s: Detection of the DNA (genes) ?

Squamous epithelial cells should be negative for IL26/CD3E



Expression of PDL1 RNA (brown dots) in human lung cancer tissue, RNA in situ hybridization (ISH) using automated RNAscope® Leica Assay-BROWN

Dots in the nuclei`s`?



cancer tissue, RNA in situ hybridization (ISH)

Volume 49(5): 603–611, 2001
The Journal of Histochemistry & Cytochemistry
http://www.jhc.org

ARTICLE

Single-copy Gene Detection Using Branched DNA (bDNA) In Situ Hybridization

Audrey N. Player,[1] Lu-Ping Shen, Daryn Kenny, Vincent P. Antao, and Janice A. Kolberg

Bayer Diagnostics, Emeryville, California

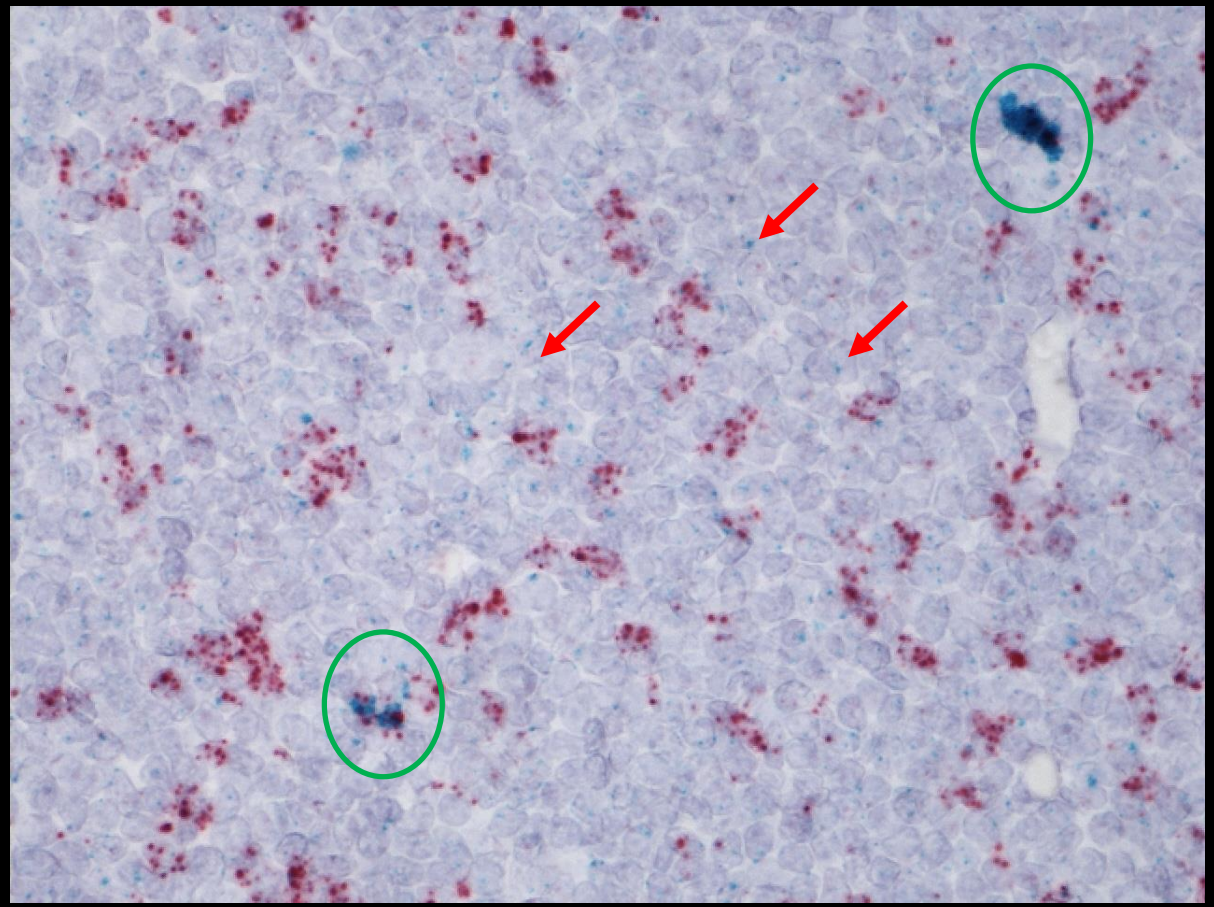
Single dots in the nuclei: Are we detecting the genes ?

SUMMARY We have developed a branched DNA in situ hybridization (bDNA ISH) method for detection of human papillomavirus (HPV) DNA in whole cells. Using human cervical cancer cell lines with known copies of HPV DNA, we show that the bDNA ISH method is highly sensitive, detecting as few as one or two copies of HPV DNA per cell. By modifying sample pretreatment, viral mRNA or DNA sequences can be detected using the same set of oligonucleotide probes. In experiments performed on mixed populations of cells, the bDNA ISH method is highly specific and can distinguish cells with HPV-16 from cells with HPV-18 DNA. Furthermore, we demonstrate that the bDNA ISH method provides precise localization, yielding positive signals retained within the subcellular compartments in which the target nucleic acid sequences are localized. As an effective and convenient means for nucleic acid detection, the bDNA ISH method is applicable to the detection of cancers and infectious agents. (J Histochem Cytochem 49:603–611, 2001)

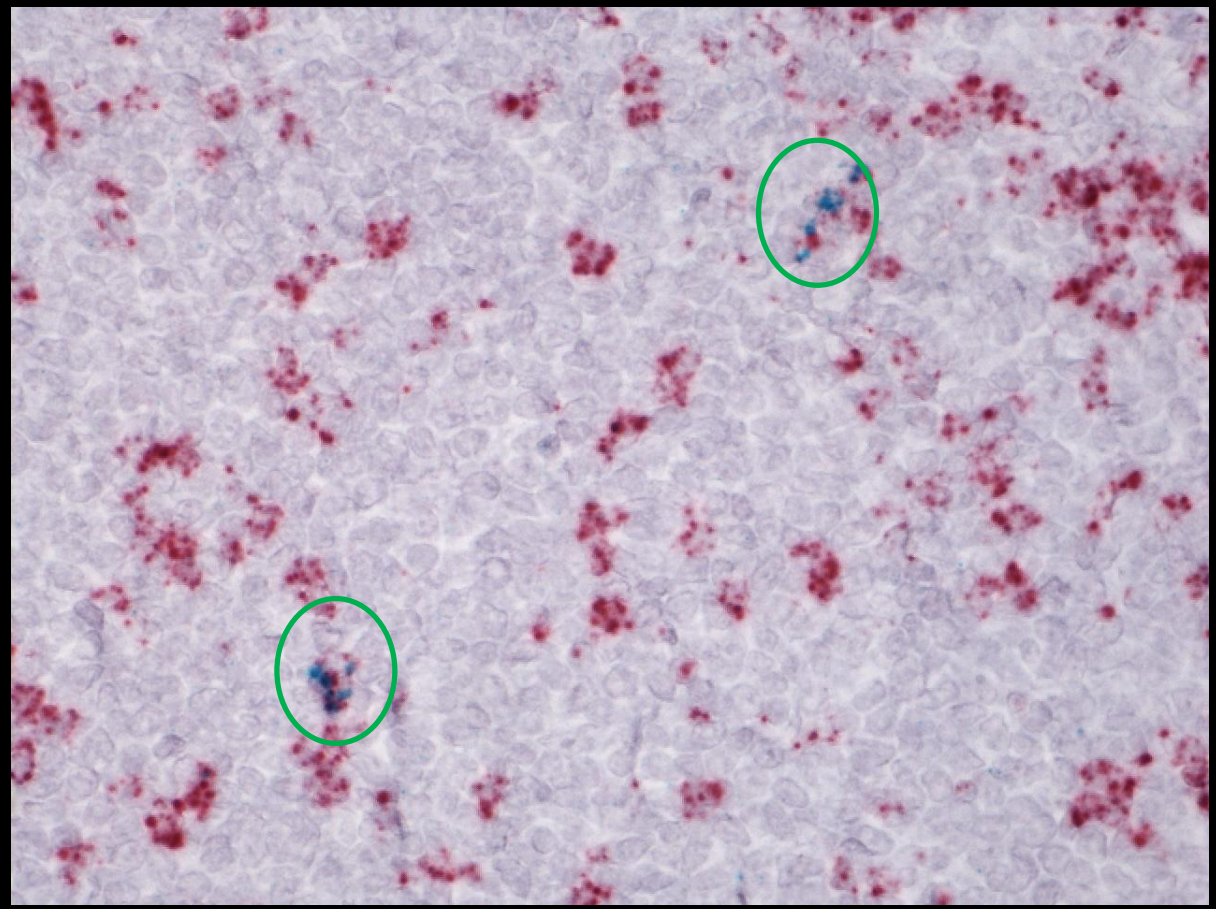
KEY WORDS
branched DNA (bDNA) signal amplification
in situ hybridization (ISH)
cervical cancer cell lines
human papillomavirus (HPV)

RNAscope IL17A/CD3E: With and without a DNAse pre-treatment step

Without DNAse



With DNAse (Qiagen 4`)

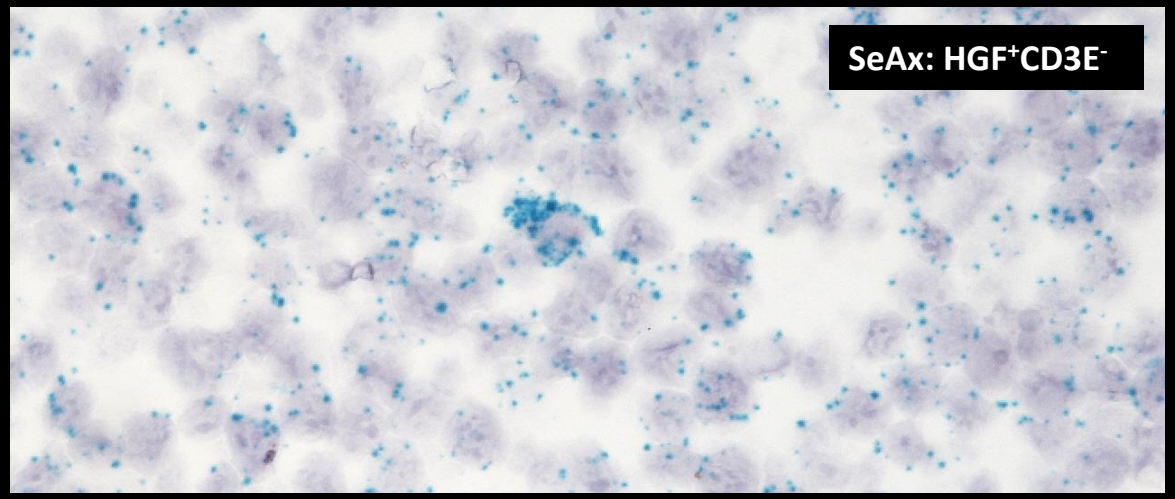


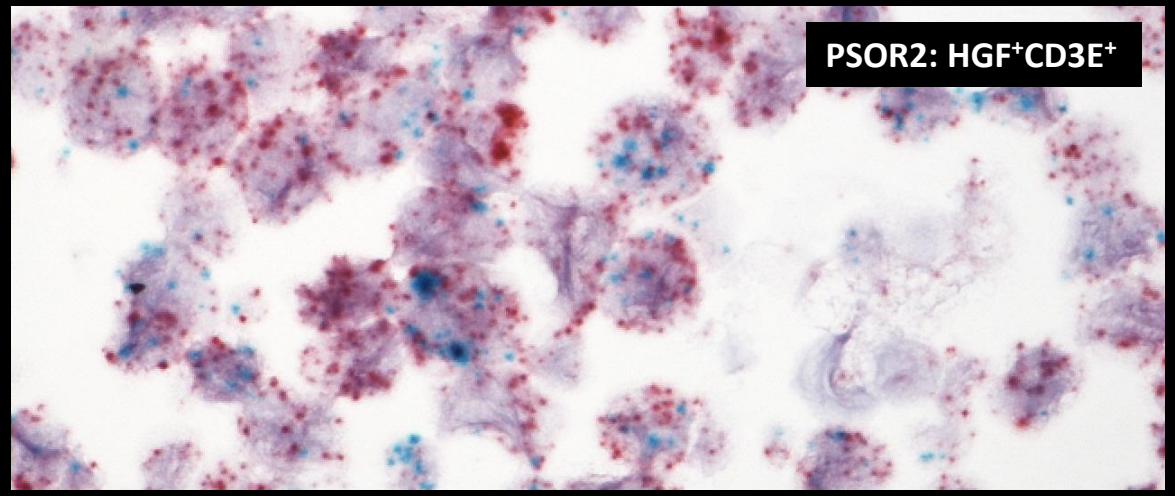
The pre-treatment with DNAse eliminated reactions related to dots in the nuclei`s. Specific signals are preserved.

bDNA In situ hybridization (RNAscope)

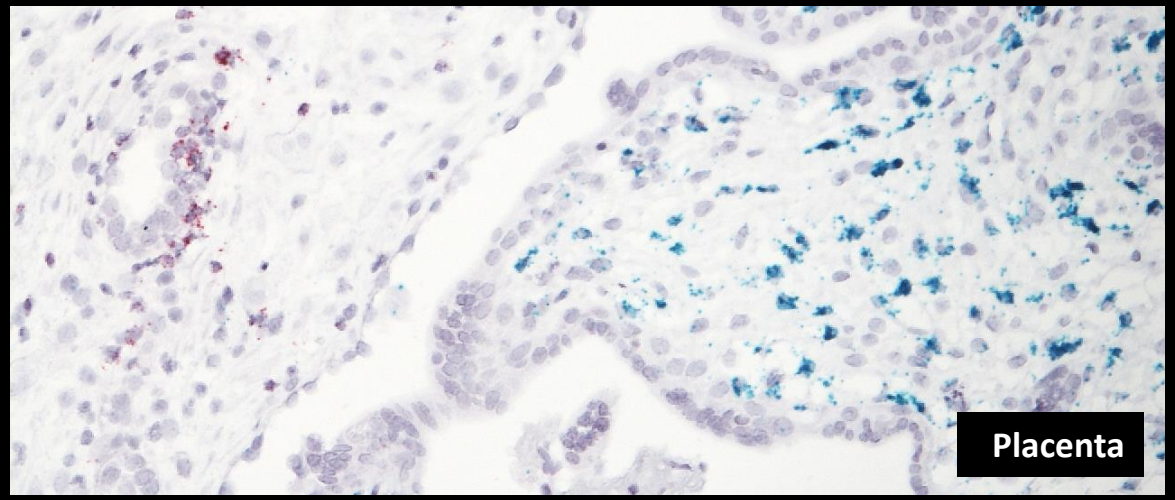
Summary:

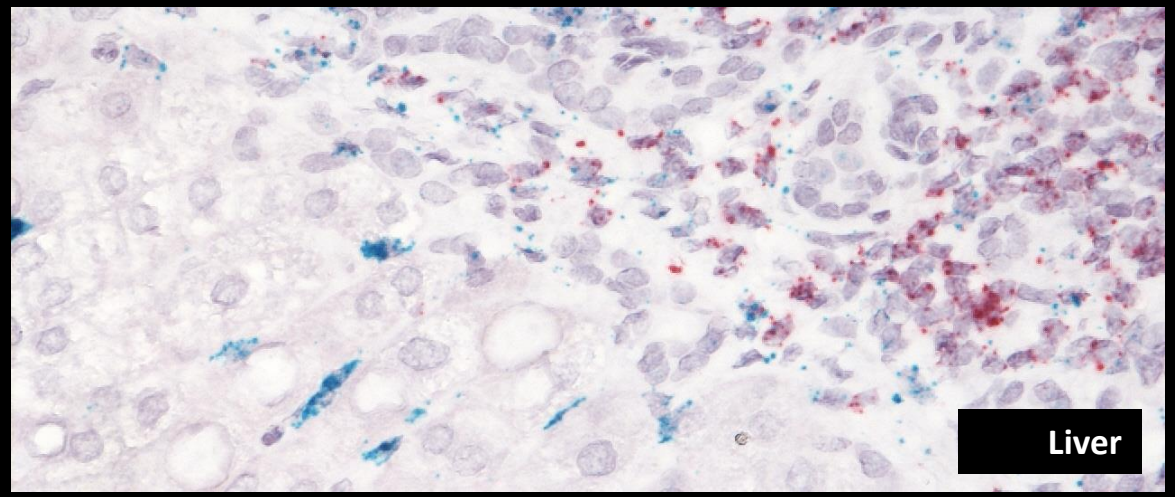
- It works , especially with the SinglePlex assay
    - Based on C1 probes (DAB) and single detection reagents
- Using Duplex Kit - select the right channel of the probe
    - C1 for the most abundant expressed target mRNA (not always possible to predict)
    - Cross-reactivity and false positive result (mixed color) may be seen
- Single mRNA molecule detection - be critical ?
    - E.g., single “nuclear dots” could be the gene
- ViewRNA ?



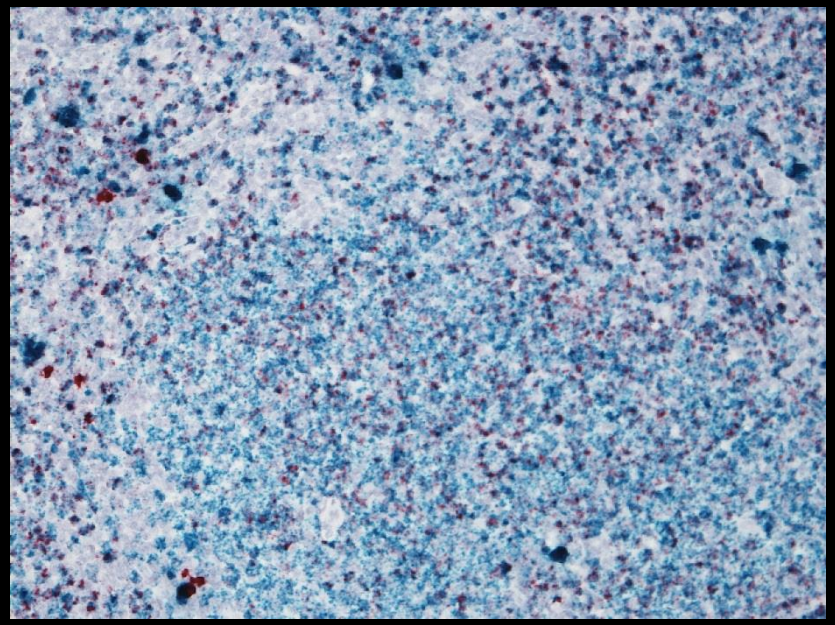
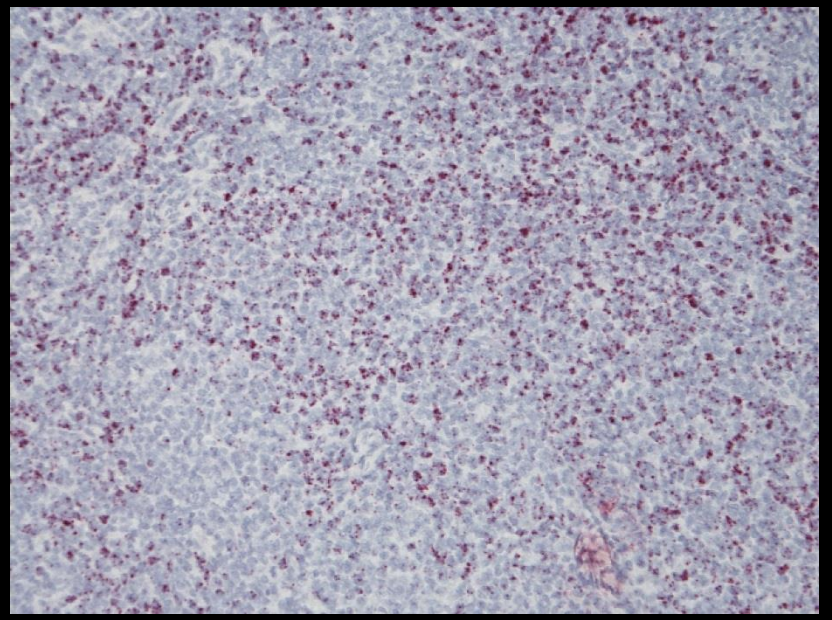
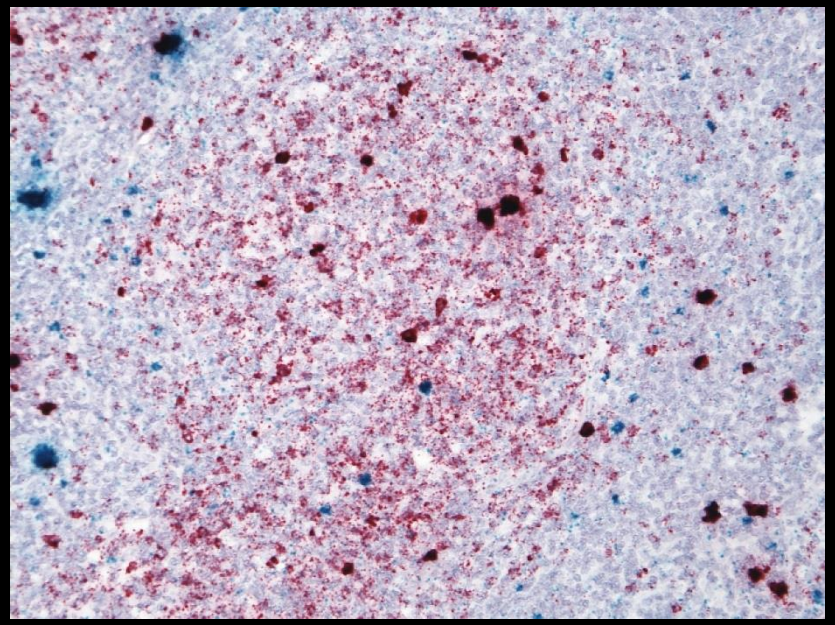
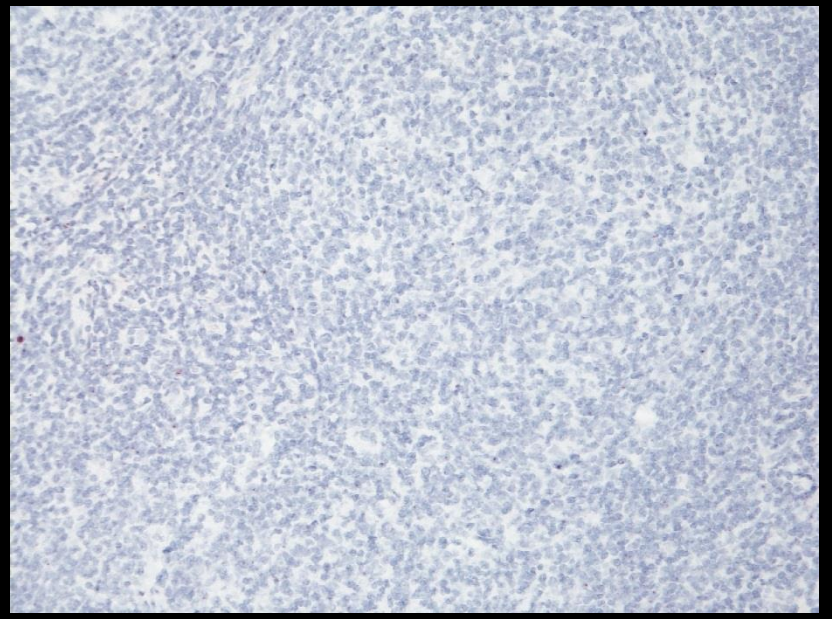


Thank you for your attention

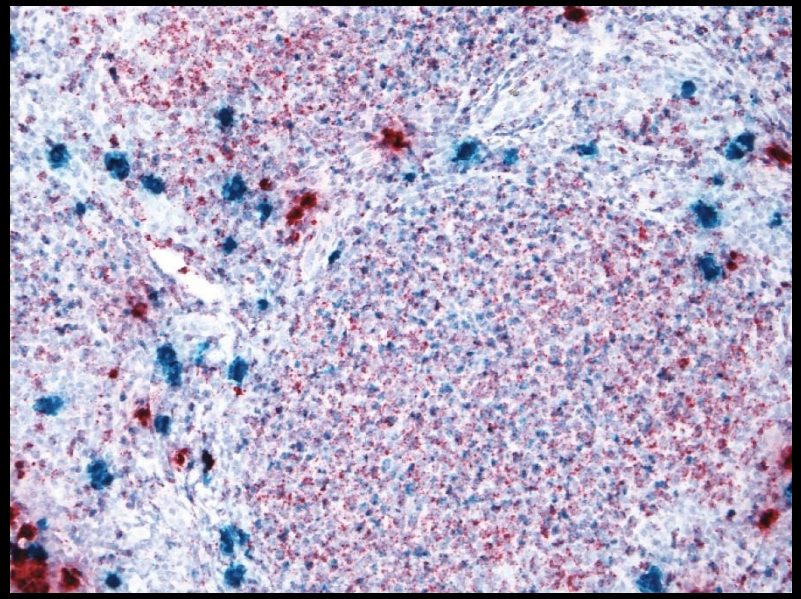
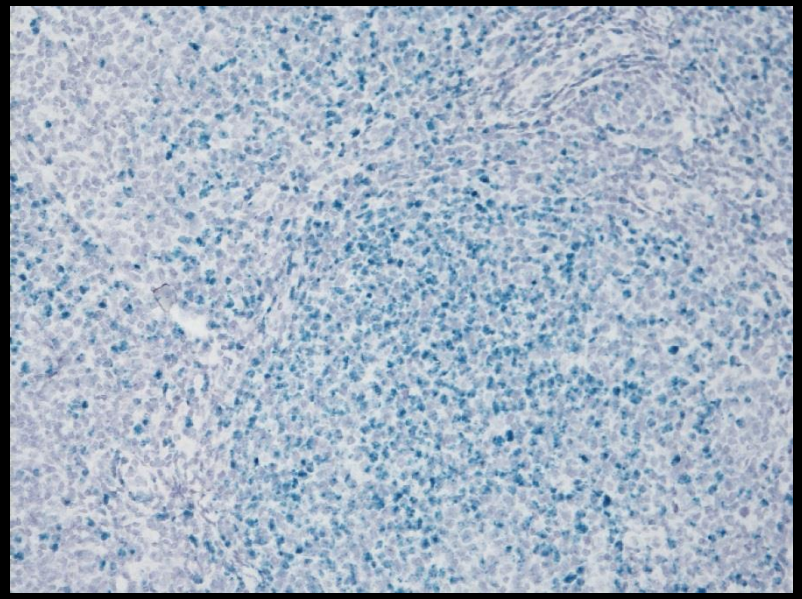
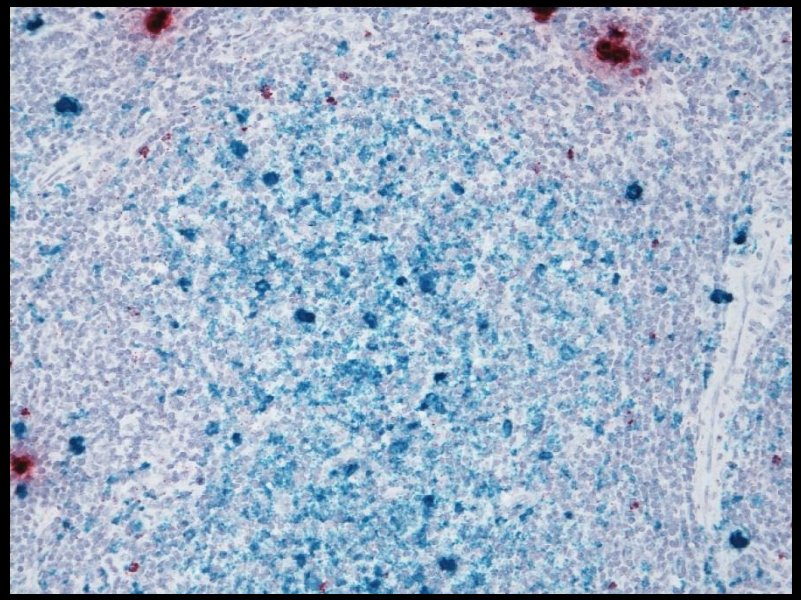
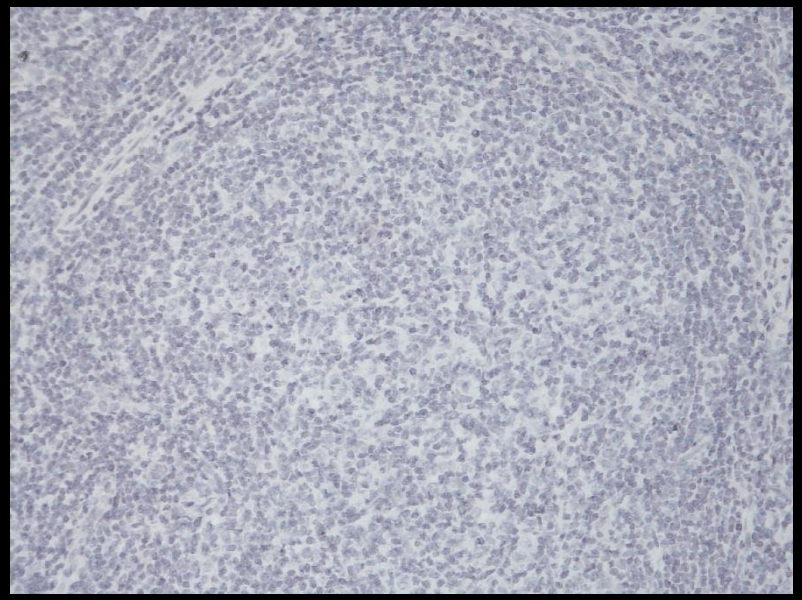




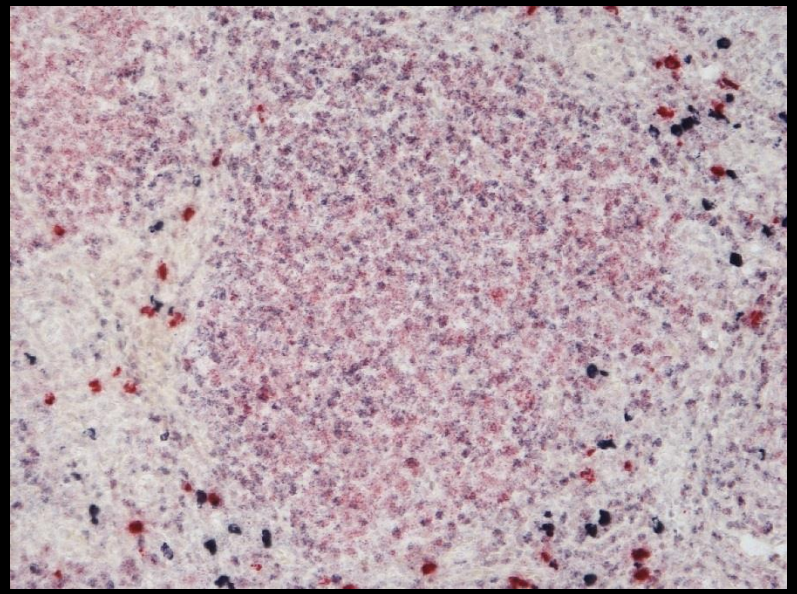
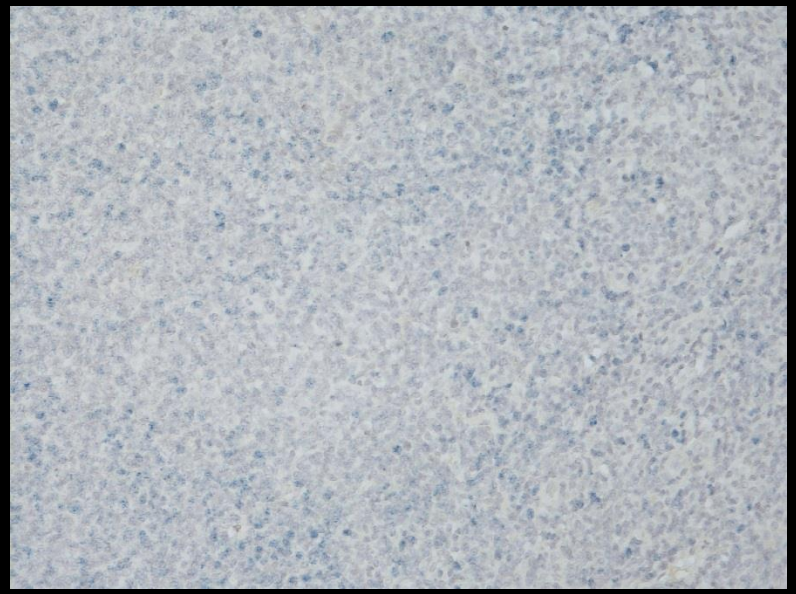
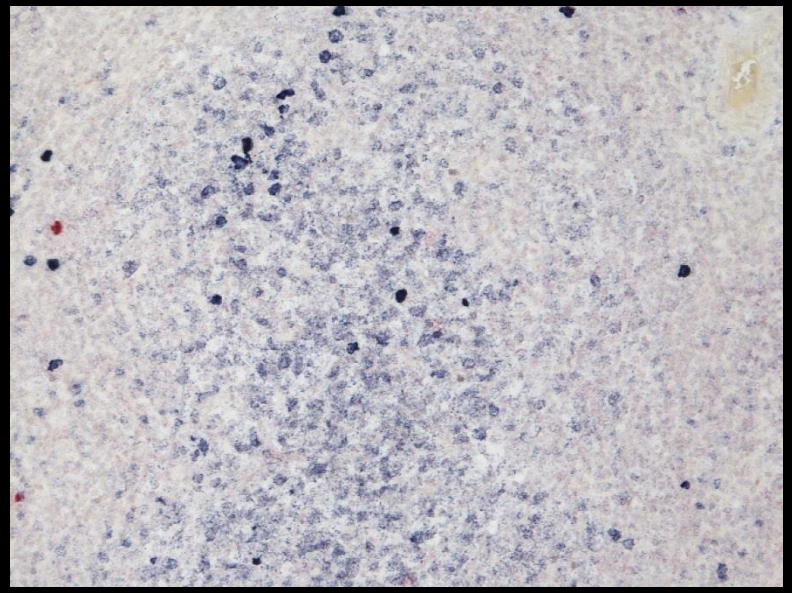
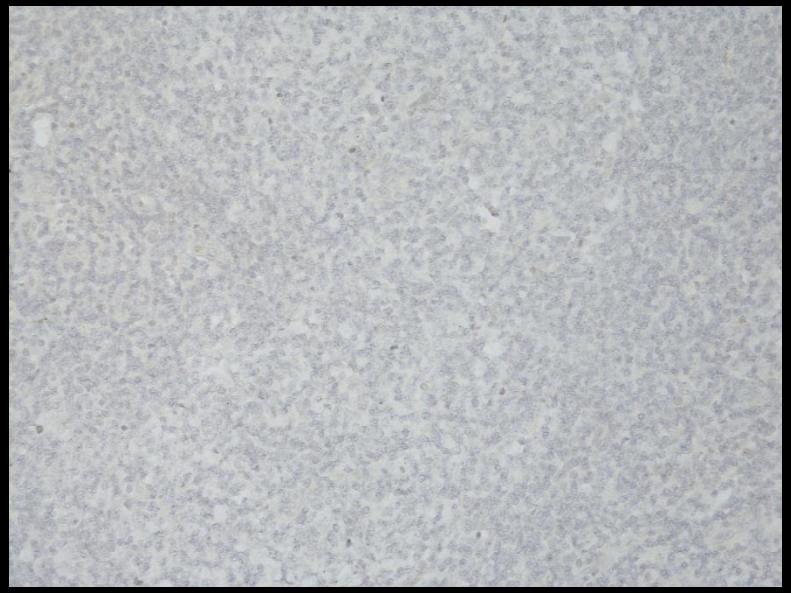
BaseScope (Duplex)

	BaseScope (Kappa/C1 + Lambda/C2)	BaseScope (IGLL5/C2)
Follicular Lymphoma (Kappa+/Green)		
Follicular Lymphoma (Lambda+/Red)		

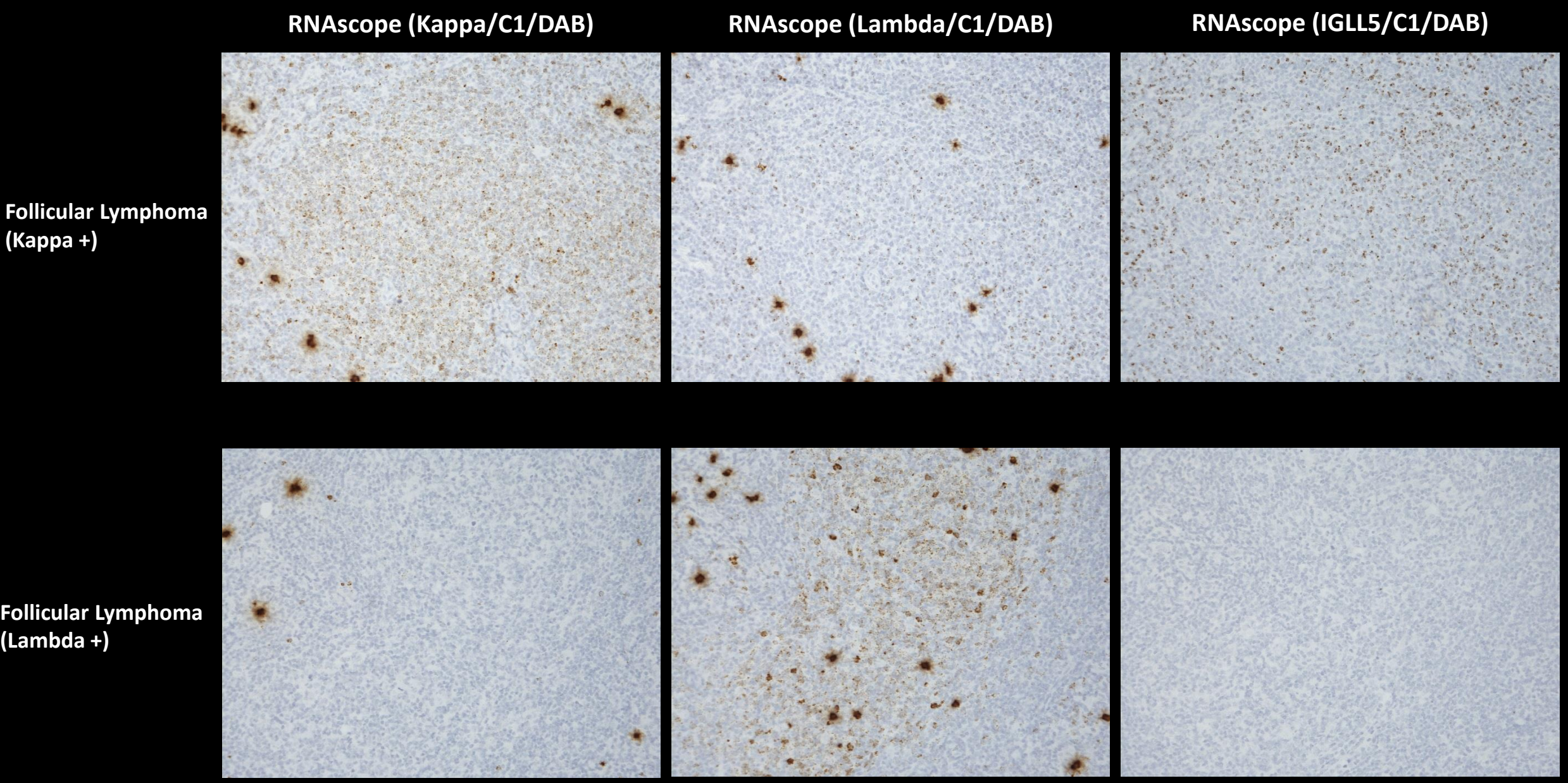
RNAscope (Duplex)

	RNAscope (Kappa/C2 + Lambda/C1)	RNAscope (IGLL5/C1)
Follicular Lymphoma (Kappa+/Red)		
Follicular Lymphoma (Lambda+/Green)		

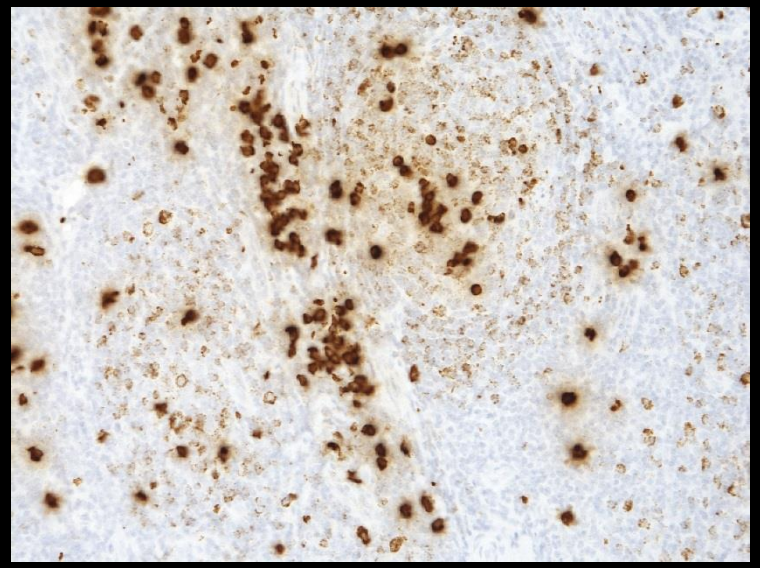
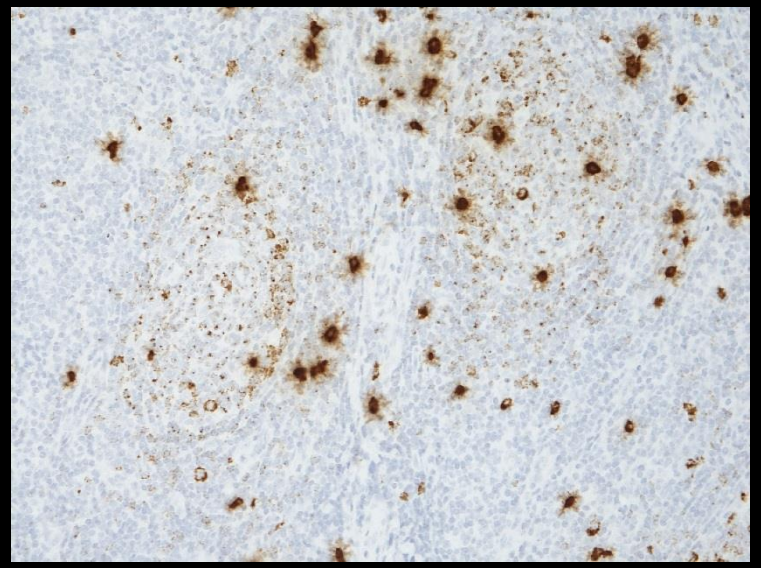
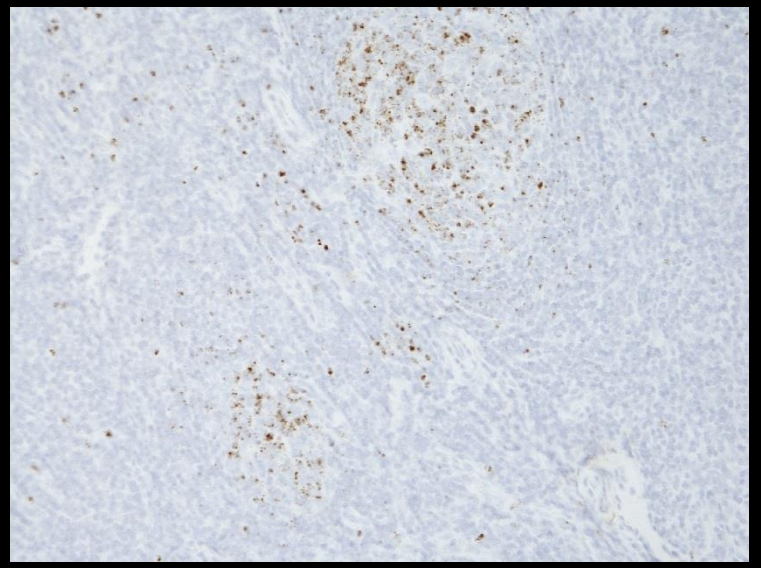
ViewRNA (Duplex)

	ViewRNA (Kappa/T1 + Lambda/T6)	ViewRNA (IGLL5/T6)
Follicular Lymphoma (Kappa+/Red)		
Follicular Lymphoma (Lambda+/Blue)		

RNAscope (SinglePlex)



RNAscope (SinglePlex)

	RNAscope (Kappa/C1/DAB)	RNAscope (Lambda/C1/DAB)	RNAscope (IGLL5/C1/DAB)
Tonsil (x20)			
Tonsil (x40)