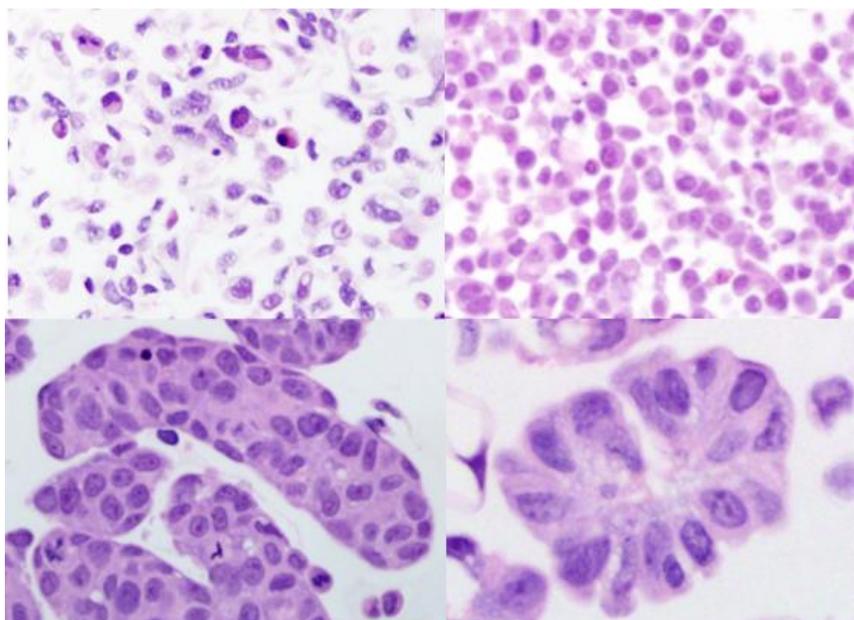


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HistoCyte Laboratories Ltd is based in the heart of the Newcastle University campus. Started in 2014 by scientists with a combined experience of over 30 years in the development of reagents for immunohistochemistry and in-situ hybridization. Collaborating with pathologists locally and globally, HistoCyte Laboratories Ltd is developing a range of cost effective products designed to help scientists to maintain and develop the quality of assays within their laboratory.



1. What is ALK?

Anaplastic lymphoma kinase (ALK) is a tyrosine kinase receptor encoded by the *ALK* gene. Synonyms include:

- CD246
- C2orf2
- EMAP-4
- ELP120
- NBLST3

Little is known of the specific function of ALK, however, it's understood to have a role in embryogenesis and the early development of the brain by regulating the proliferation of nerve cells.¹ In adults its expression is restricted to a few organs including brain, testis, small intestine, prostate and colon.²

2. Role of ALK in Cancer

The ALK gene is found on the short arm of chromosome 2. As an oncogene it was first identified as a translocation in anaplastic large cell lymphoma (ALCL) t(2;5)(p23;q35). In this instance the translocation caused a fusion product with the nucleophosmin gene: NPM-ALK.² In reality the ALK translocation is a promiscuous event and associated with numerous fusions in multiple malignancies, see tables 1 and 2.³

Table 1. Chromosomal translocation and fusion proteins in solid tumours involving ALK gene.

Disease	Chromosomal rearrangement	Fusion protein	Frequency (%)
NSCLC	inv(2)(p21;p23)	EML4-ALK	2-5
	t(2;3)(p23;q21)	TFG-ALK	2
	t(2;10)(p23;p11)	KIF5B-ALK	<1
	t(2;14)(p23;q32)	KLC1-ALK	<5
	t(2;9)(p23;q31)	PTPN3-ALK	ND
IMT	t(1;2)(q25;p23)	TPM3-ALK	0.5
	t(2;19)(p23;p13)	TPM4-ALK	<5
	t(2;17)(p23;q23)	CLTC-ALK	<5
	inv(2)(p23;q35)	ALK-ATIC	<5
	t(2;11;2)(p23;p15;q31)	CARS-ALK	<5
BC	t(2;2)(p23;q13)	RANBP2-ALK	<5
	inv(2)(p23;p15;q31)	RANBP2-ALK	<5
	t(2;4)(p23;q21)	SEC31L1-ALK	<5
	inv(2)(p21;p23)	EML4-ALK	<5
	inv(2)(p21;p23)	EML4-ALK	<5
CRC	t(2;2)(p23.3)	C2orf44-ALK	<5
	t(2;19)(p23;p13)	TPM4-ALK	ND
ESCC	t(2;19)(p23;p13)	TPM4-ALK	ND
RCC	t(2;10)(p23;q22)	VCL-ALK	ND
	t(1;2)(q25;p23)	TPM3-ALK	ND
	inv(2)(p21;p23)	EML4-ALK	ND

NSCLC; non-small cell lung cancer. IMT; inflammatory myofibroblastic tumour, BC; breast cancer, CRC; colorectal cancer, ESCC; esophageal squamous cell carcinoma, RCC; renal cell carcinoma. ND: not determined.

Most recently, therapy for the fusion EML4-ALK in lung cancer has created fresh focus on the detection of ALK but in relation to non-small cell lung cancer (NSCLC)^{3,4,5} rather than NPM-ALK, which has long been used in the diagnosis of ALCL.

Table 2. Chromosomal translocations and fusion proteins in hematologic malignancies involving ALK gene.

Disease	Chromosomal rearrangement	Fusion protein	Frequency (%)
ALCL	t(2;5)(p23;q35)	NPM-ALK	75-80
	t(2;17)(p23;q25)	ALO17-ALK	<1
	t(2;3)(p23;q21)	TFG-ALK	2
	t(2;X)(p32;q11-q12)	MSN-ALK	<1
	t(1;2)(q25;p23)	TPM3-ALK	Dec-18
	t(2;19)(p23;p13)	TPM4-ALK	<1
	inv(2)(p23;q35)	ATIC-ALK	2
	t(2;22)(p23;q11.2)	MYH9-ALK	<1
	t(2;17)(p23;q23)	CLTC-ALK	2
	DLBCL	t(2;5)(p23;q35)	NPM-ALK
t(2;17)(p23;q23)		CLTC1-ALK	ND
t(2;5)(p23.1;q35.3)		SQSTM1-ALK	ND
HL	ins(4)(2;4)(p23;q21)	SQSTM1-ALK	ND
	t(2;4)(p24;q21)	SEC31A-ALK	ND
	t(2;5)(p23;q35)	NPM-ALK	ND

ALCL; anaplastic large cell lymphoma, DLBCL; diffuse large B cell lymphoma; HL; Hodgkin lymphoma; ND; not determined

3. Detecting EML4-ALK

Until recently, ALK translocations in NSCLC have been detected by either polymerase chain reaction (PCR) or visually assessed by fluorescence in situ hybridisation (FISH). For some time it was believed that immunohistochemistry (IHC) was not sensitive enough for the detection of EML4-ALK. However, with the re-optimization of some of the currently available antibodies and the advent of new clones and detection systems, IHC is becoming more widely adopted.

The most commonly used antibodies on the market, clones 5A4, D5F3 and ALK1,⁶ all recognise C-terminus (see respective vendor data sheets), the green sections in Figure 1 below. This is the conserved ALK region harbouring the tyrosine kinase domain. Therefore, all of these antibodies should recognise the ALK fusion proteins. In practice the efficacy of the antibody or its affinity for the target epitope, the relative availability of fusion protein and appropriate epitope retrieval and IHC protocols mean that variation is seen from laboratory to laboratory. This is evidenced in external quality assurance programs.^{6,7}

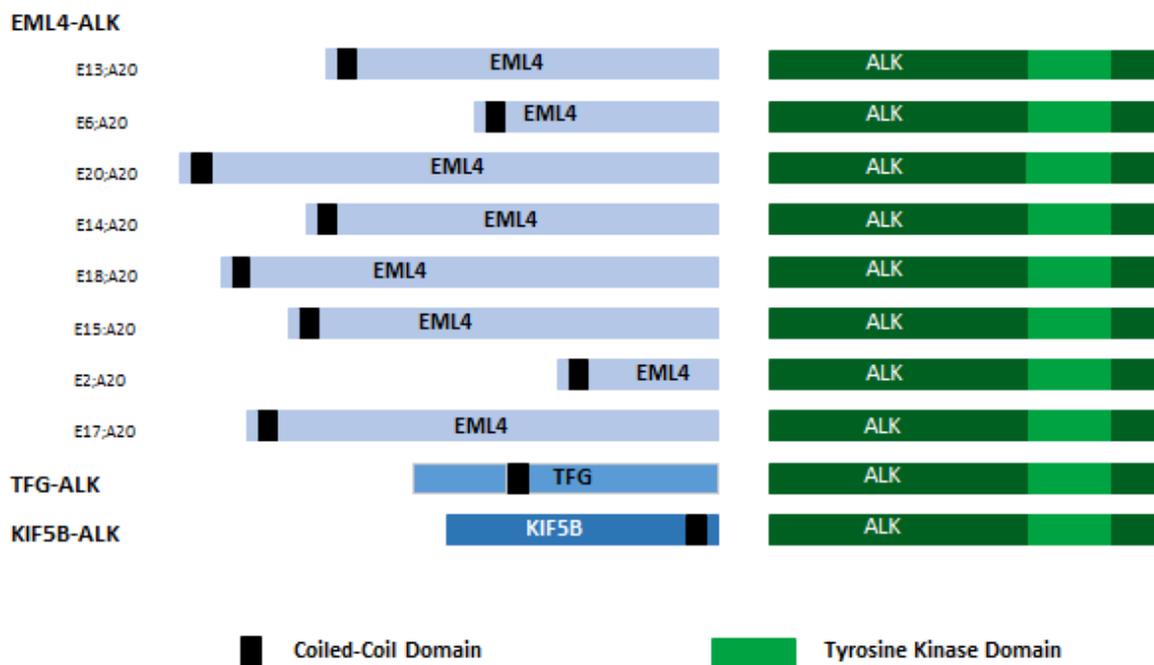


Figure 1. Different variants of EML4-ALK and non-EML4 fusion partners. The nomenclature refers to the exon in EML4 translocated to the exon in ALK (adapted from reference 5).

4. Cell Line Controls

Both ALK products are sold in two formats. Pre-prepared slides as in figure 2 below.

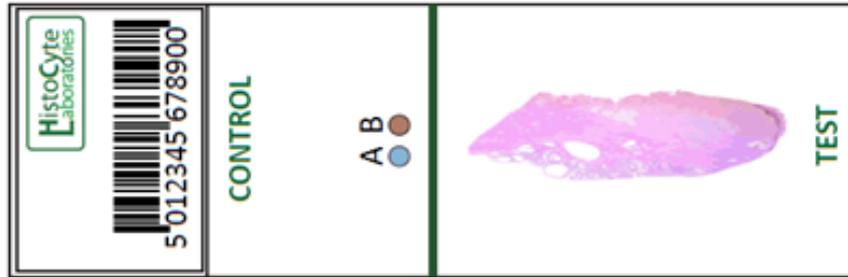


Figure 2: Cell Line Control Slide

Or in a cell microarray (CMA) paraffin wax block as illustrated in figure 3.

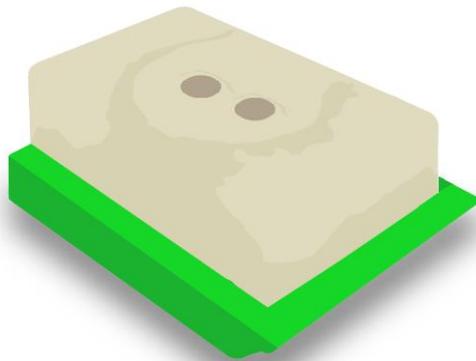


Figure 3: CMA block

Both formats have their merit depending on the needs of the laboratory. The slides offer ease of use and save time in preparation. However, in high volume centers the blocks provide a more cost effective solution and fit into the work flow of the laboratory easily.

In either case the analyte controls demonstrate that the reagents employed to perform the assay have worked effectively in combination with the staining protocol. They determine:

- Reagent performance
- Correct implementation of the staining protocol (manual or automated)

They confer confidence i.e. those reviewing the slide can be reassured that if the control has worked appropriately, the assay has worked and any staining present within the sample is genuine.

The expression patterns of the 2 cell lines for both ALK-Lung and ALK-Lymphoma are shown in table 3a and 3b below:

Cell Lines	ALK Gene Status	ALK-Lung
A	Negative	Negative
B	ALK translocation positive	Positive For EML4-ALK

Table 3a. Cell status for ALK-Lung.

Cell Lines	ALK Gene Status	ALK-Lymphoma
A	Negative	Negative
B	ALK translocation positive	Positive For NPM-ALK

Table 3b. Cell status for ALK-Lymphoma.

4.1 Product summary and codes

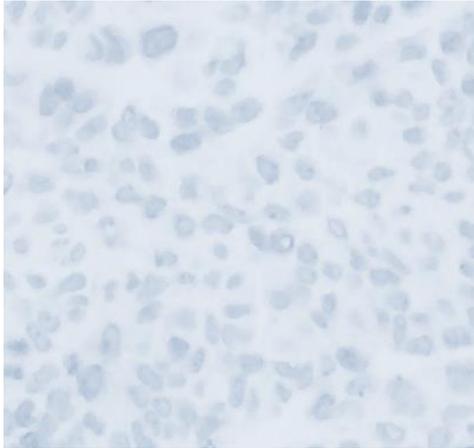
	Product Name	Format	Code
ALK	ALK-Lung Analyte Control (Two core positive and negative for the EML4-ALK translocation)	Slide(2)	HCL007
		Slide(5)	HCL008
		Block	HCL009
	ALK-Lymphoma Analyte Control (Two core positive and negative for the NPM-ALK translocation)	Slide(2)	HCL010
		Slide(5)	HCL011
		Block	HCL012

5. Expected Staining Results for ALK-Lung

The following section gives micrographs of the expected results obtained with each of the cells assessed by IHC with the standardised companion diagnostic from Roche/Ventana and FISH assay from Abbott Molecular. For more information please email info@histocyte.com

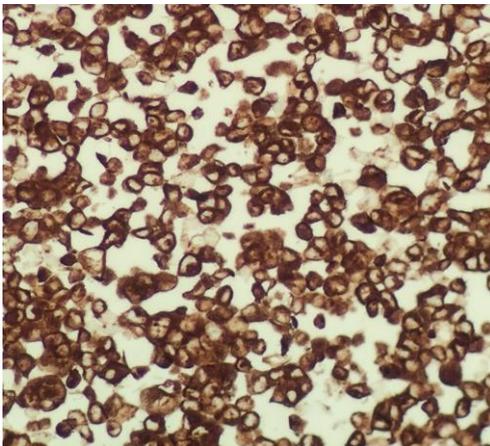
5.1 ALK-Lung harbouring ALK-EML4 fusion protein. Immunohistochemistry: Roche/Ventana, anti-ALK (D5F3) Rabbit Monoclonal Primary Antibody. (790-4843)

A



No staining present.

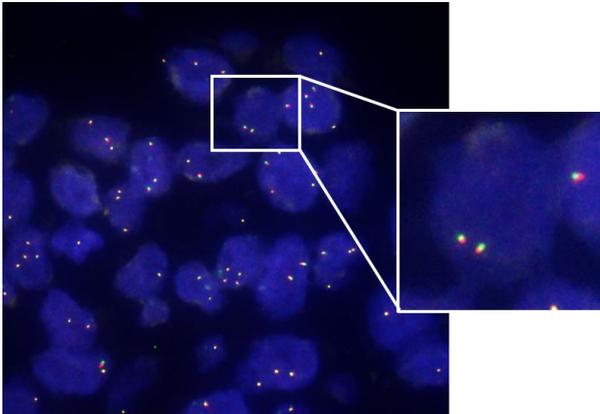
B



>95% of cells demonstrate intense cytoplasmic staining.

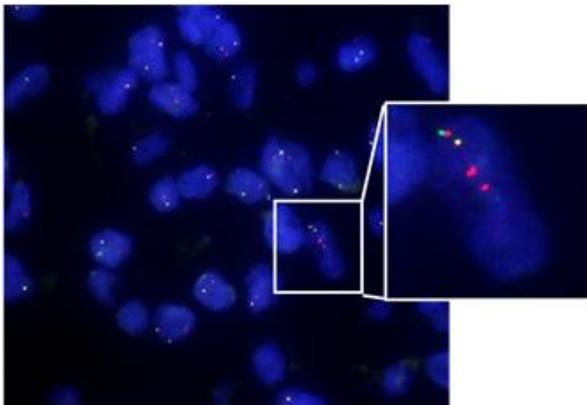
5.2 ALK translocation Fluorescence in situ hybridization: Abbott Molecular, Vysis ALK Break Apart FISH Probe Kit. 06N38-020.

A



Signals overlapped or clustered together <2 signals apart.

B



Green and red signals clearly split. >2 signal diameters apart of one another.

6. Troubleshooting

The Roche/Ventana assay is known to produce non-specific cytoplasmic punctate staining. It is documented in the VENTANA ALK Scoring Interpretation Guide for non-small cell lung carcinoma (NSCLC).⁸ These observations have been seen in the negative cell line in the course of assessment. Its occurrence is erratic and due to the amplification steps associated with the detection system Optiview™. The Interpretation guide from Roche/Ventana is clear that weak diffuse cytoplasmic staining should be considered negative. Figure 4 below shows an example of this in a negative cell sample.

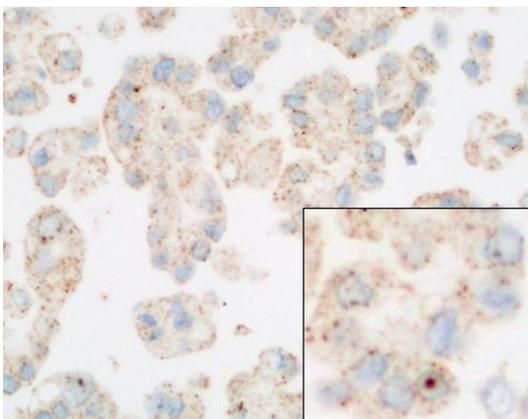


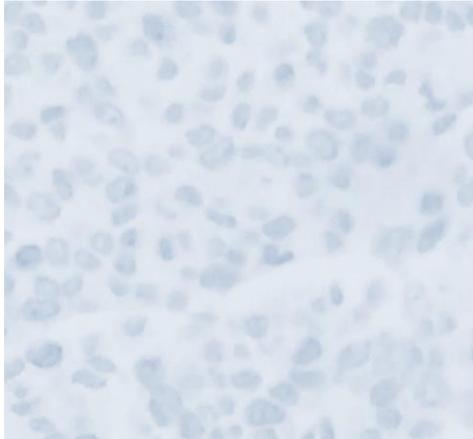
Figure 4. Non-specific cytoplasmic staining, note the larger deposits (bottom of insert). This should be considered negative.

7. Expected staining results for ALK-Lymphoma

The following section gives micrographs of the expected results obtained with each of the cells assessed by IHC with Confirm Anti-ALK (ALK01) primary antibody from Roche/Ventana. For more information please email info@histocyte.com

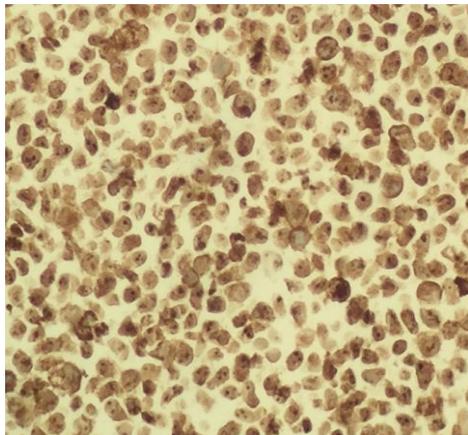
7.1 ALK-Lymphoma harbouring ALK-NPM fusion protein. Immunohistochemistry: Roche/Ventana, anti-ALK (ALK01) Mouse Monoclonal Primary Antibody. (790-2918)

A



No staining present.

B



>95% of cells demonstrate intense cytoplasmic staining.

8. References

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- 8) VENTANA ALK Scoring Interpretation Guide for non-small cell lung carcinoma (NSCLC). 1011879EN, October 2012, Revision D.

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