HistoCyte Laboratories

Quality in Control

ALK-Lung Analyte Control (EML4-ALK)
ALK-Lymphoma Analyte Control (NPM-ALK)
ALK Analyte Control^{DR}

Product Codes:

ALK-Lung: HCL007, HCL008 and HCL009

ALK-Lymphoma: HCL010, HCL011 and HCL012

ALK Analyte Control^{DR}: HCL053, HCL054 and HCL055

Issue 1. 220314

Contents

Product Name	Format	Code
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
ALK Analyte Control^{DR} (Four cores: negative, positive for WT ALK, positive for EML4-ALK and positive for NPM-ALK)	Slide (2)	HCL053
	Slide (5)	HCL054
	Block	HCL055

(For research use only)

Introduction to ALK

What is it?

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]

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 indiscriminate event and associated with numerous fusions in multiple malignancies, see tables 1 and 2.[3]

1. Roskoski R Jr. Anaplastic lymphoma kinase (ALK): structure, oncogenic activation, and pharmacological inhibition. Pharmacol Res. 2013 Feb;68(1):68-94.
2. Morris SW, Kirstein MN, Valentine MB, Dittmer K, Shapiro DN, Look AT, et al. Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. Science 1994;263:1281-4
3. Iragavarapu C, Mustafa M, Akinleye A, Furqan M, Mittal V, Cang S, Liu D. Novel ALK inhibitors in clinical use and development. J Hematol Oncol. 2015 Feb 27;8(1):17

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)(p23q32)	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
	t(2;2)(p23;q13)	RANBP2-ALK	<5
	inv(2)(p23;p15;q31)	RANBP2-ALK	<5
	t(2;4)(p23;q21)	SEC31L1-ALK	<5
BC	inv(2)(p21;p23)	EML4-ALK	<5
CRC	inv(2)(p21;p23)	EML4-ALK	<5
	t(2;2)(p23.3)	C2orf44-ALK	<5
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.

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)	CLTCL-ALK	2
DLBCL	t(2;5)(p23;q35)	NPM-ALK	ND
	t(2;17)(p23;q23)	CLTC1-ALK	ND
	t(2;5)(p23.1;q35.3)	SQSTM1-ALK	ND
	ins(4)(2;4)(p23;q21)	SQSTM1-ALK	ND
	t(2;4)(p24;q21)	SEC31A-ALK	ND
HL	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

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.

Detecting EML-4 ALK

In the past 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.

3. Iragavarapu C, Mustafa M, Akinleye A, Furqan M, Mittal V, Cang S, Liu D. Novel ALK inhibitors in clinical use and development. J Hematol Oncol. 2015 Feb 27;8(1):17
4. Shaw AT, Kim DW, Nakagawa K, Seto T, Crinó L, Ahn MJ, De Pas T, Besse B, Solomon BJ, Blackhall F, Wu YL, Thomas M, O'Byrne KJ, Moro-Sibilot D,Camidge DR, Mok T, Hirsh V, Riely GJ, Iyer S, Tassell V, Polli A, Wilner KD, Jänne PA.Crizotinib versus chemotherapy in advanced ALK-positive lung cancer.N Engl J Med. 2013 Jun 20;368(25):2385-94.
5. Sasaki T, Rodig SJ, Chirieac LR, Jänne PA. The Biology and Treatment of EML4-ALK Non-Small Cell Lung Cancer. Eur J Cancer. 2010 July ; 46(10): 1773–1780.

The most commonly used antibodies on the market, clones 5A4, D5F3 and ALK1[6]. Other clones such as 1A4 have also made it into routine use in recent years. All of these clones recognise epitopes on the 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]

The availability of the ALK Analyte ControlDR enables the user to determine if the assay is sensitive enough for use in ALCL or NSCLC. This product contains both EML4-ALK and NPM-ALK, the latter is typically expressed at significantly higher levels compare to EML4-ALK. This product also has a WT ALK expressing cell line, this really allows one to determine the assays lower levels of ALK detection – giving significant confidence regarding the assays suitable sensitivity.

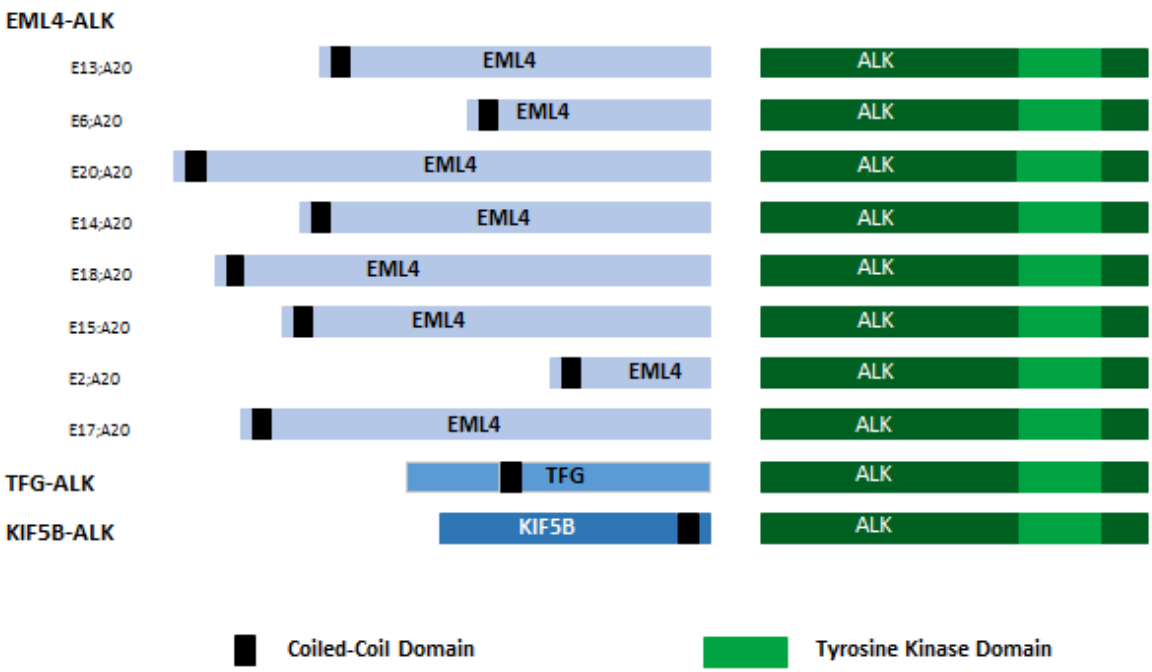


Figure 1. Different variants of EML4-ALK and non-EML4 fusion partners. The nomenclature refers to the exon in EML4 translocated to the exon in ALK (adapted from reference 5).

6. UKNEQAS Journal: Immunocytochemistry. Run 108/37. Assessments Dates: 5th-23rd January 2015.http://www.ukneqasicc.ucl.ac.uk/run_108_journal.pdf
7. NordiQC Lung Anaplastic Lymphoma Kinase (lu-ALK) Assessment Run 39 2013 http://www.nordiqc.org/Run-39-B16-H4/Assessment/Run39_ALK.pdf

Quality Control

One of the requirements of quality standardization is the appropriate use of controls. These need to be robust enough for IHC and in situ hybridization (ISH), be reproducible and cost-effective. Additionally, the control material should be consistent from batch to batch and throughout the block it is cut from.

Same slide control versus batch controls

In laboratories with automated platforms these controls need to be on the same slide. Batch controls are typically not representative anymore of how slides have been treated as the instruments treat the slides completely independently.

External Quality Assurance

External quality assurance (EQA) schemes or proficiency testing (PT) have shown standardized assays typically perform better than laboratory developed tests (LDTs). In 2017 over 60% of UKNEQAS participants in RUN118/47 were using standardized ER vendor assays. Again in the NordiQC assessment B25 in 2018 >80% were using standardized ER assays.

Cell Lines as Controls

The issue with tissue

Laboratories often struggle for appropriate control tissue. Not only is it hard to find tissue in sufficient amount, but also biomarker expression can vary throughout tissue, often due to a number of factors including but not limited to:

- Fixation
- Processing artefact
- Heterogeneity of the protein, see figure 3 (taken from Nitta H *et al*[8])

This means that tissue selected for use as control can vary to the point that it makes its use as a control redundant.

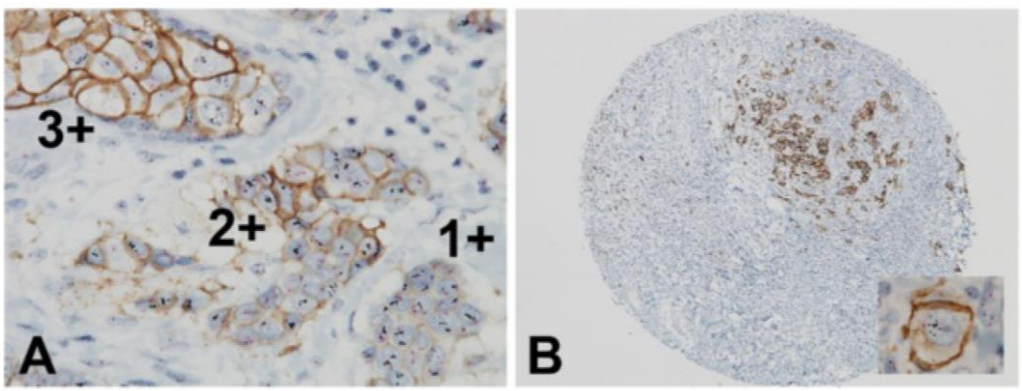


Figure 2. Results of HER2 gene-protein staining of FFPE breast cancer tissues exhibiting heterogeneity of HER2 positive tumor cell populations or isolated tumour cell populations. (A) The HER2 gene-protein assay demonstrated the heterogeneity of HER2 positive tumour cell populations in FFPE breast cancer tissues. In the sample shown, cell populations with HER2 IHC scores of 3+, 2+ and 1+ neighbor each other and all tumor populations present amplified *HER2* gene. However, the HER2 IHC 3+ tumor cell population contains dispersed *HER2* gene copies while the HER2 IHC 2+ and 1+ population contains clustered *HER2* gene copies [40x]. **(B)** The HER2 gene-protein assay clearly visualized small groups of HER2 3+IHC breast cancer cells [4x]. The insert shows an isolated individual HER2 IHC positive tumor cell with *HER2* gene amplification [100x].

Cell lines

Cell lines are typically included in or with assays as pre-cut slides. There are not enough for use as same slide and pre-cut slides do not lend themselves to fitting into the work flow of the laboratory. They are also used by EQA schemes as standardized materials for their assessments.

So while adequately performing by IHC or FISH, the preparations are often sparse and the cellular integrity or morphology is generally poor. So while they can be reproducibly manufactured providing standardized material there is room for improvement.

Our solution

HistoCyte Laboratories cell lines are compact and typically "tissue-like". In particular the breast ductal carcinoma cells often create "pseudo-acini" producing a more tissue like appearance. The morphology of our cells means that they can tell you more about how they have been treated. It is quite obvious when the morphology is disrupted.

The HistoCyte Laboratories cell lines are intended to be used for quality control only. They are standardized so are developed and manufactured to provide consistent results throughout the block. This is what differentiates them from tissue controls.

Tissue is still important

It is important to remember that these are a quality control material designed only to demonstrate that the assay has worked consistently. They reduce the burden on a laboratory to identify and obtain suitable materials for use as a same slide control. This means tissue can be preserved for other uses such as trouble shooting and validations.

ALK-Lung Analyte Control

The **ALK-Lung Analyte Control** is sold in two formats: as pre-prepared slides (Figure 3) or as a cell microarray (CMA) paraffin wax block (Figure 4).

Figure 3: Cell Line Control Slide

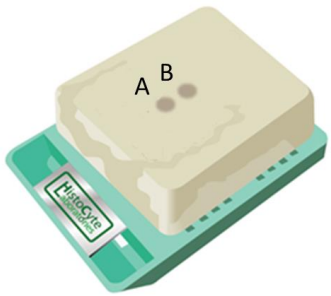


Figure 4: CMA block

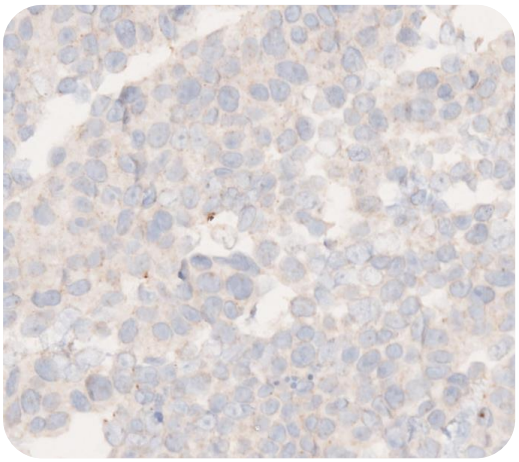
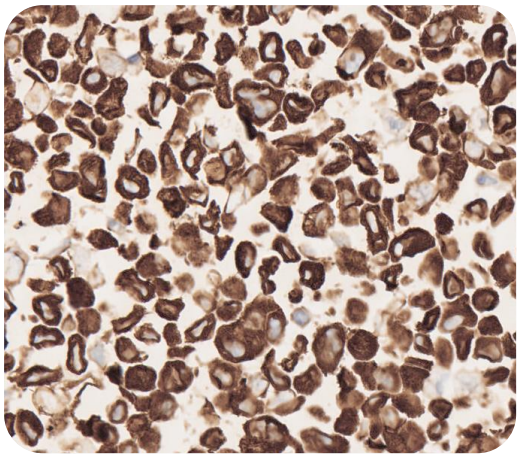
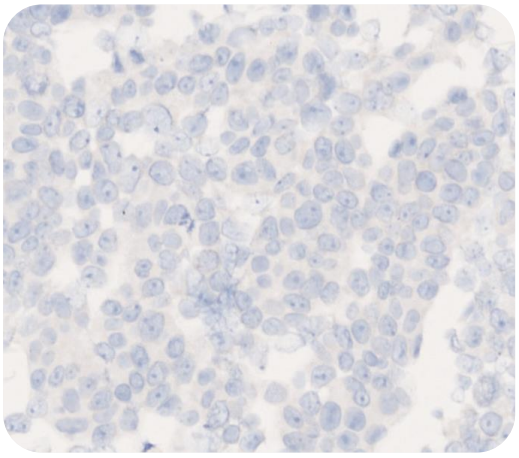
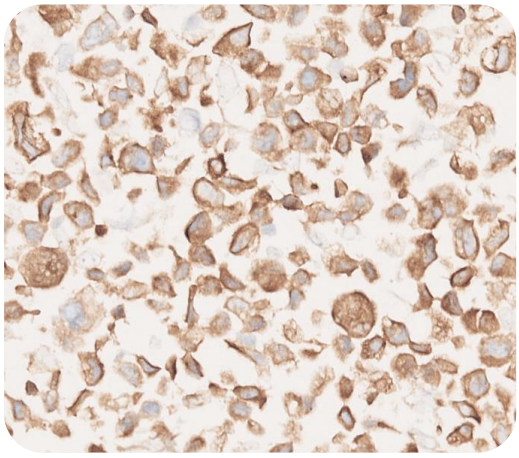
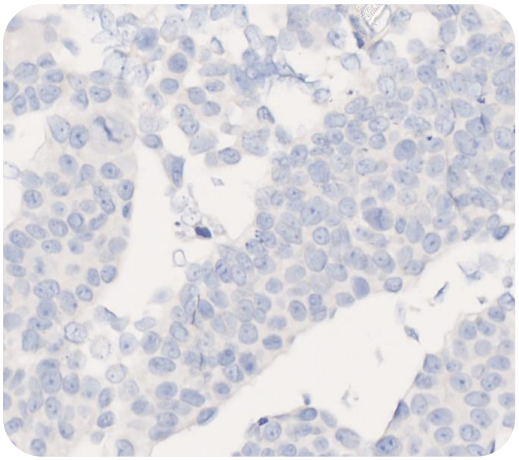
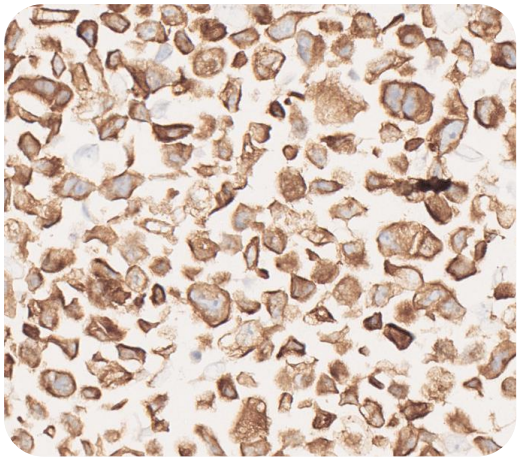
Our CMA block provides the most cost effective solution for high volume centres. They have been purposely designed to fit seamlessly into the work flow of the laboratory.

Our pre-prepared slides offer a ready-to-go alternative that saves time in preparation. These are ideal for one-off assessments, research laboratories and preliminary product trials.

The expression patterns of the 2 cell lines for ALK-Lung are shown below:

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

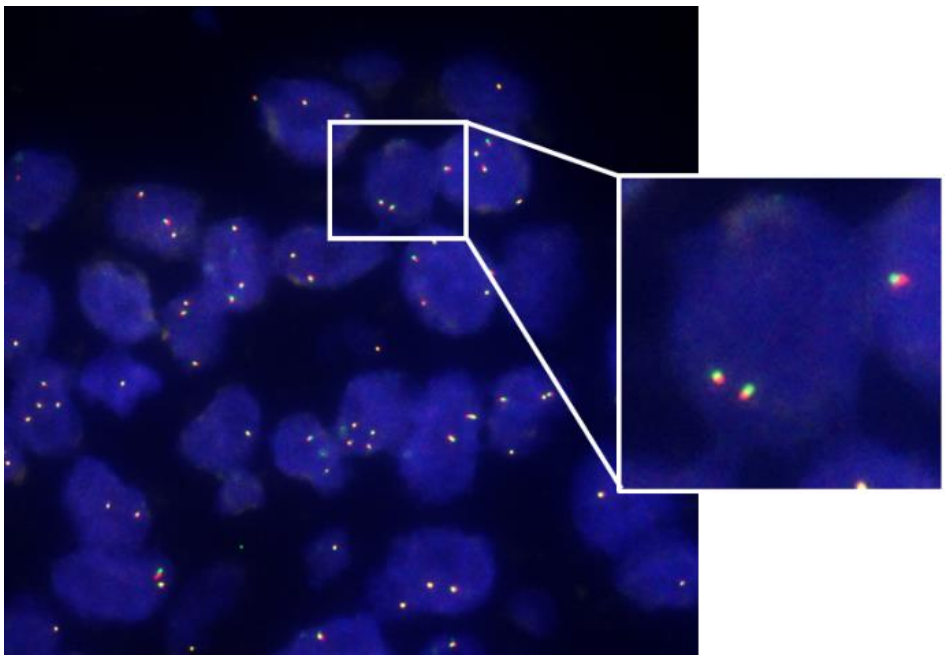
ALK-Lung Analyte Control IHC

	Breast adenocarcinoma **A**	Lung Adenocarcinoma **B**
Roche/Ventana, anti-ALK (D5F3) Rabbit Monoclonal Primary Antibody. (790-4843)		
Cell Signalling Technologies Clone D5F3 at a 1/20 dilution on the Ventana Benchmark Ultra (No Amp)		
Origene Clone 1A4 at a 1/320 dilution on the Ventana Benchmark Ultra (No Amp)		

ALK-Lung Translocation Fluorescence *in situ* Hybridization

A

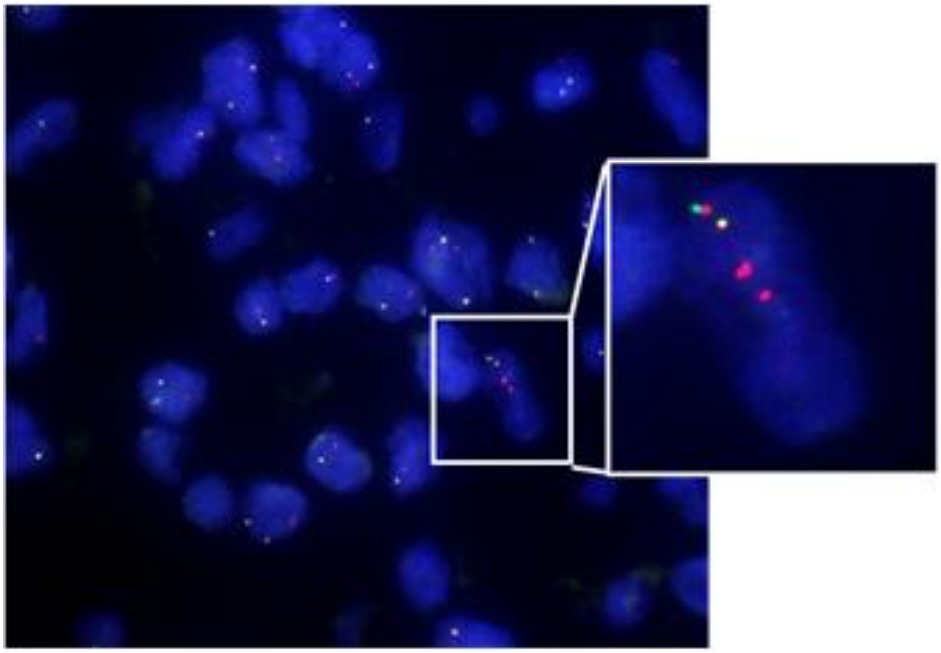
Breast adenocarcinoma



Signals overlapped or clustered together <2 signals apart

B

Lung Adenocarcinoma



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

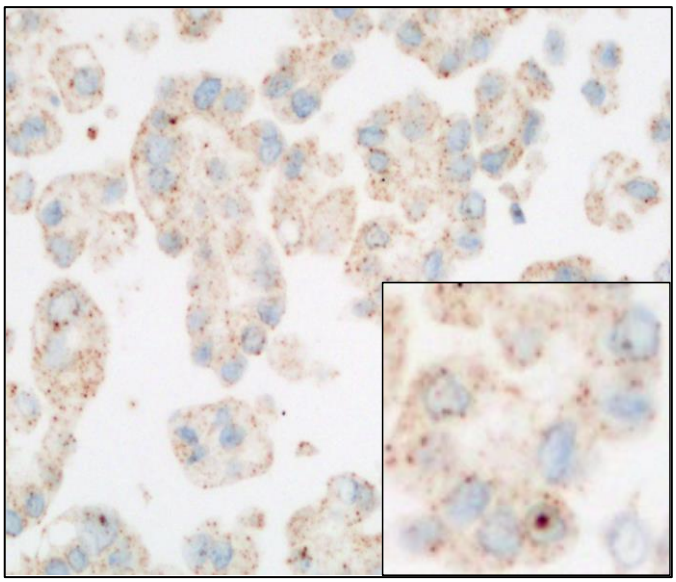
Abbott Molecular, Vysis ALK Break Apart FISH Probe Kit. 06N38-020

ALK-Lung 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).[9] 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. The image below shows an example of excessive background in a negative cell sample (cell line A).

A

Breast adenocarcinoma



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

Roche/Ventana, anti-ALK (D5F3) Rabbit Monoclonal Primary Antibody. (790-4843)

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

ALK-Lymphoma Analyte Control

The **ALK-Lymphoma Analyte Control** is sold in two formats: as pre-prepared slides (Figure 5) or as a cell microarray (CMA) paraffin wax block (Figure 6).

Figure 5: Cell Line Control Slide

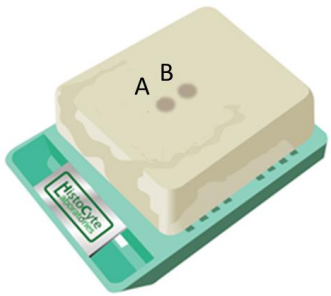


Figure 6: CMA block

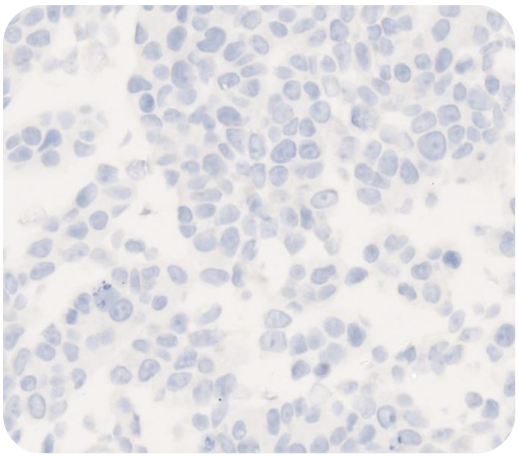
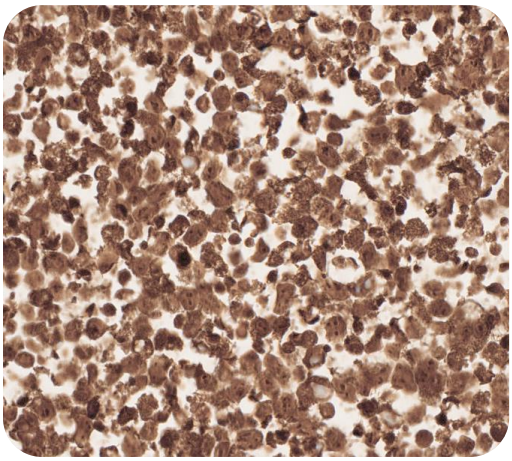
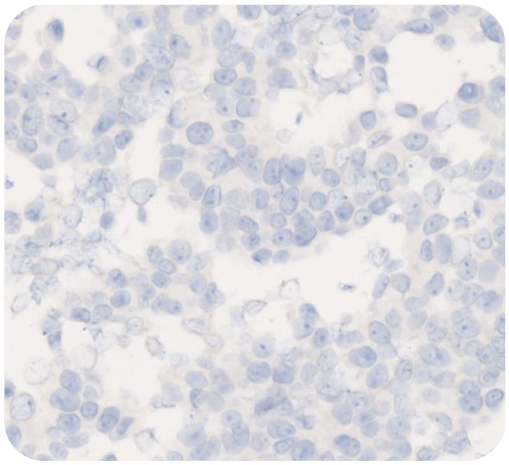
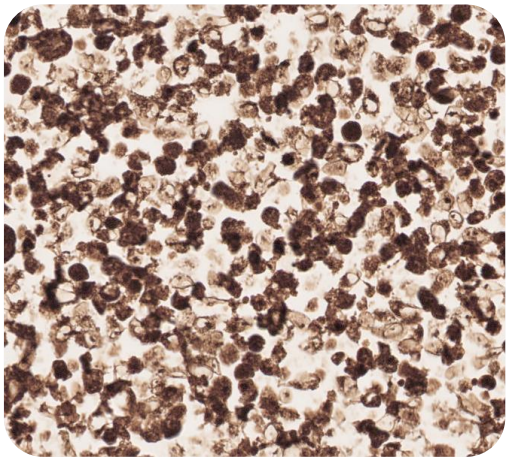
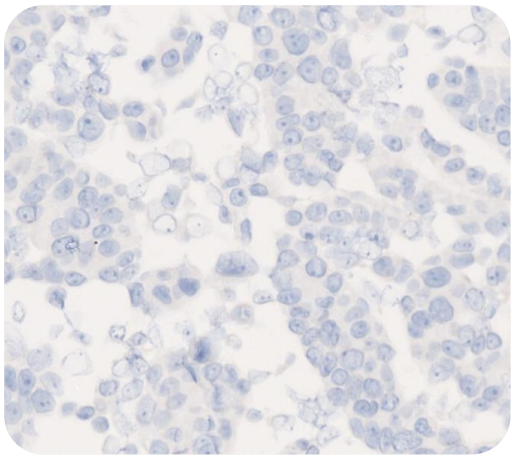
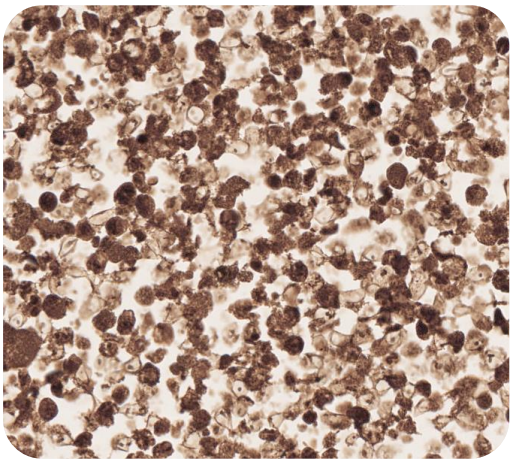
Our CMA block provides the most cost effective solution for high volume centres. They have been purposely designed to fit seamlessly into the work flow of the laboratory.

Our pre-prepared slides offer a ready-to-go alternative that saves time in preparation. These are ideal for one-off assessments, research laboratories and preliminary product trials.

The expression patterns of the 2 cell lines for ALK-Lymphoma are shown below:

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

ALK-Lymphoma Analyte Control IHC

	Breast adenocarcinoma **A**	Lung Adenocarcinoma **B**
Roche/Ventana, anti-ALK (ALK01) Rabbit Monoclonal Primary Antibody. (790-2918)		
Cell Signalling Technologies Clone D5F3 at a 1/320 dilution on the Ventana Benchmark Ultra		
Origene Clone 1A4 at a 1/1280 dilution on the Ventana Benchmark Ultra		

ALK Analyte Control^{DR}

The **ALK Analyte Control^{DR}** is sold in two formats: as pre-prepared slides (Figure 7) or as a cell microarray (CMA) paraffin wax block (Figure 8).

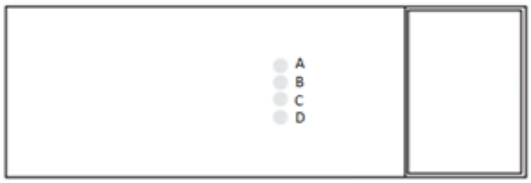


Figure 7: Cell Line Control Slide

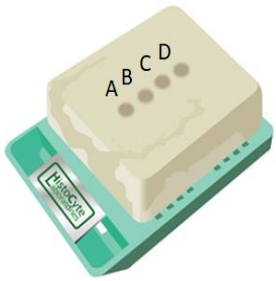


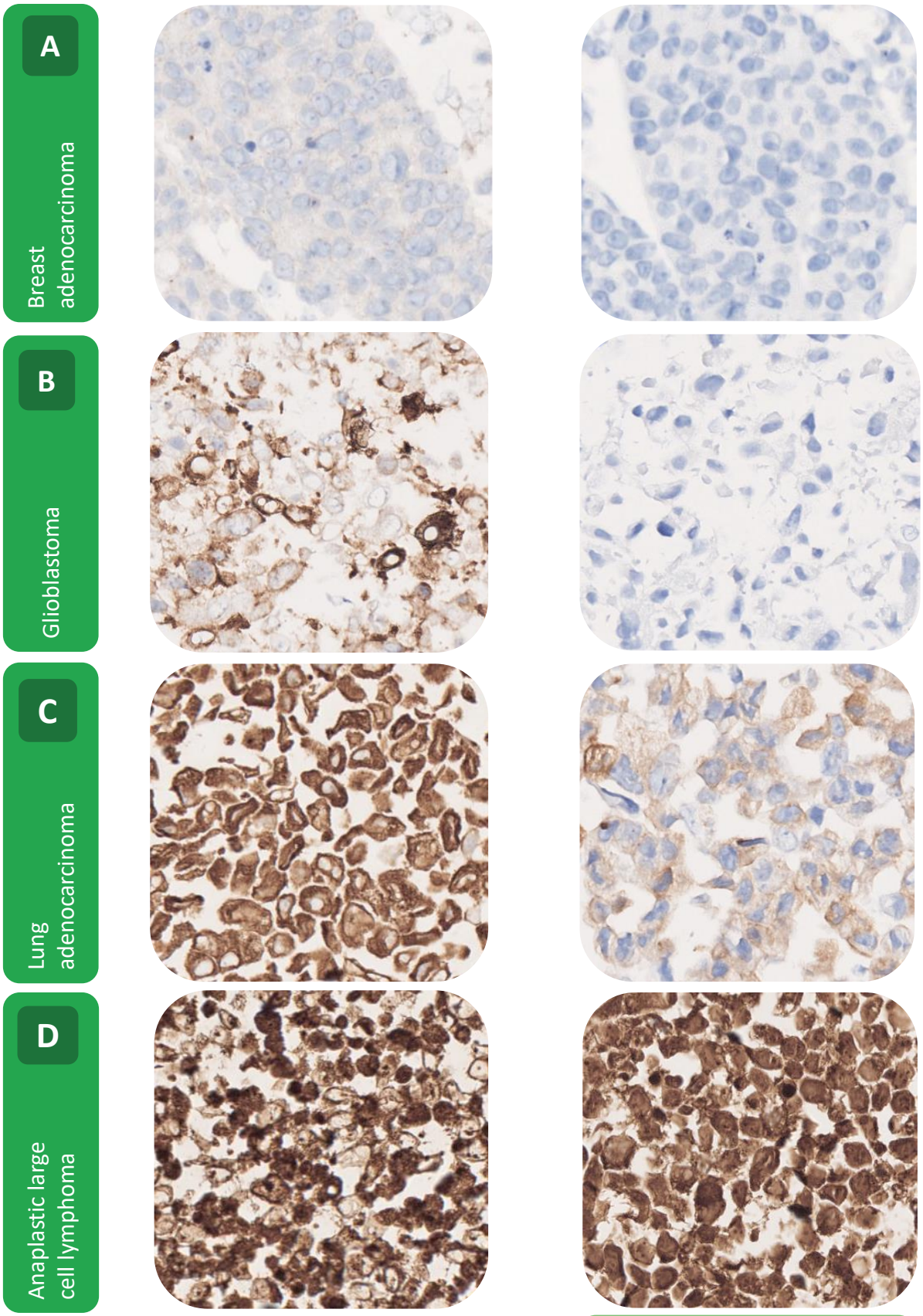
Figure 8: CMA block

Our CMA block provides the most cost effective solution for high volume centers. They have been purposely designed to fit seamlessly into the work flow of the laboratory.

The ALD Dynamic Range product allows the user to determine if the ALK assay being used is sensitive enough for use in the detection of EML4-ALK, NPM-ALK or both. The expression patterns of the 4 cell lines for the ALK Dynamic Range control are shown below:

Cell line	IHC for ALK-EML4 (Lung)	IHC for ALK-NPM (Lymphoma)	FISH for ALK-NPM (Lymphoma)
A Negative	Negative	Negative	Negative
B WT ALK	Low	Negative	Negative
C EML4-ALK	High	Mid	Positive
D NPM-ALK	High	High	Positive

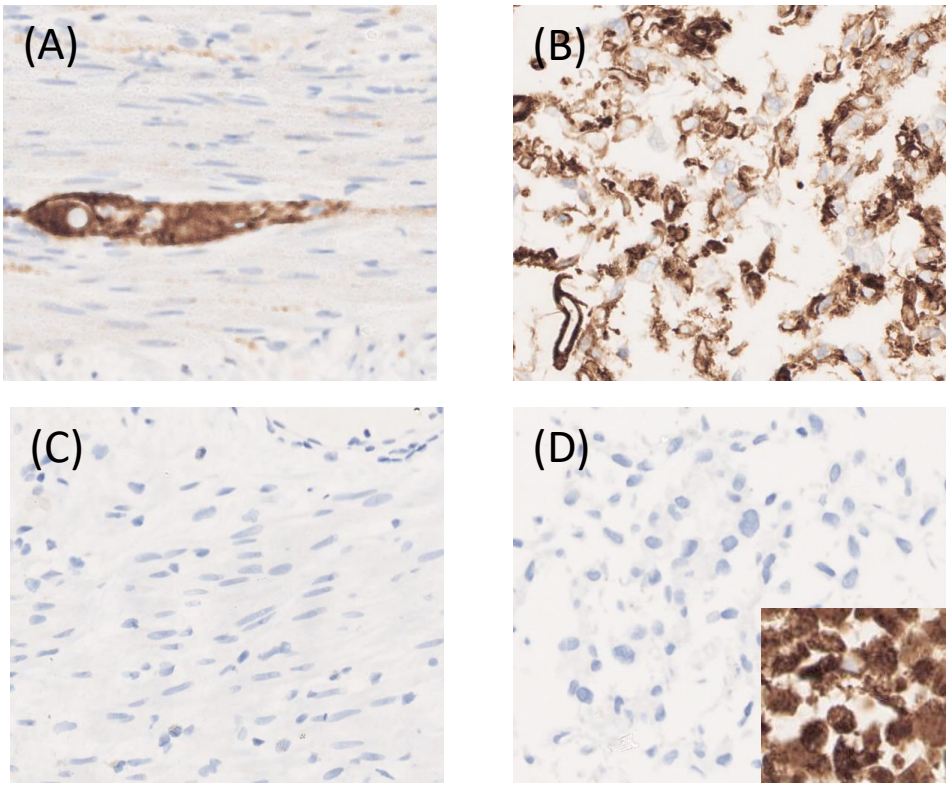
ALK Analyte Control^{DR} IHC



WT ALK and sensitivity

Wild Type ALK is expressed at low levels and demonstrating it with an ALK assay is indicative of the lower limits of ALK detection. In other words if this can be demonstrated the assay is sensitive enough to identify ALK fusion proteins that are often expressed at low levels such as EML4-ALK in NSCLC. This is why controls such as appendix are recommended by Roche with their D5F3 assay.

The cell line in core B expresses WT ALK and therefore determines the assays suitable sensitivity. The images below are the ganglia of an appendix stained along side Core B when stained with the D5F3 Assay from Roche. Where as when tested with ALK01, for lymphoma, both are negative.



Appendix (A) and Core B (B) both staining for WT ALK with the Roche D5F3 assay. Appendix (C) and Core B (D) assessed with ALK01. Inset image of (D) shows the positive control.

ALK Analyte Control^{DR} therefore allows appendix to be preserved and demonstrates suitable sensitivity for which ever ALK is being assessed.

HistoCyte Laboratories

Quality in Control

Also Available From HistoCyte Laboratories Ltd

Product Name	Format	Code
HPV/p16 Analyte Control[DR] (Four cores: negative and three positive with dynamic range of HPV gene copies)	Slide(2)	HCL001
	Slide(5)	HCL002
	Block	HCL003
HPV/p16 Analyte Control (Three cores: negative and two positive for p16 and HPV gene copies)	Slide(2)	HCL004
	Slide(5)	HCL005
	Block	HCL006
ALK-Lung Analyte Control (Two cores: negative and a positive for the EML4-ALK translocation)	Slide(2)	HCL007
	Slide(5)	HCL008
	Block	HCL009
ALK-Lymphoma Analyte Control (Two cores: negative and a positive for the NPM-ALK translocation)	Slide(2)	HCL010
	Slide(5)	HCL011
	Block	HCL012
ALK Analyte Control[DR] (Four cores: negative, positive for WT ALK, positive for EML4-ALK and positive for NPM-ALK)	Slide(2)	HCL053
	Slide(5)	HCL054
	Block	HCL055
Breast Analyte Control (Two cores: negative and positive for HER2, ER and PR)	Slide(2)	HCL013
	Slide(5)	HCL014
	Block	HCL015
Breast Analyte Control[DR] (Five cores: variable levels of expression of HER2, ER and PR. Including negative control)	Slide(2)	HCL016
	Slide(5)	HCL017
	Block	HCL018
PD-L1 Analyte Control[DR] (Four cores: negative, low, intermediate and high levels of expression of PD-L1)	Slide(2)	HCL019
	Slide(5)	HCL020
	Block	HCL021
ROS1 Analyte Control (Two cores: negative and positive for ROS1 translocation SLC34A2-ROS1)	Slide(2)	HCL022
	Slide(5)	HCL023
	Block	HCL024
ROS1 Analyte Control[DR] (Three cores: negative, FIG-ROS1 (very low fusion protein), SLC34A2-ROS1 (high fusion protein)	Slide(2)	HCL035
	Slide(5)	HCL036
	Block	HCL037
HER2 Analyte Control[DR] (Four cores: 0, 1+ (both non-amplified), 2+ (equivocal) and 3+ (amplified))	Slide(2)	HCL026
	Slide(5)	HCL027
	Block	HCL028
Estrogen Receptor Analyte Control[DR] (Four cores: negative, low, intermediate and high)	Slide(2)	HCL029
	Slide(5)	HCL030
	Block	HCL031
Progesterone Receptor Analyte Control[DR] (Four cores: negative, low, intermediate and high)	Slide(2)	HCL032
	Slide(5)	HCL033
	Block	HCL034
NTRK Analyte Control (Two cores: negative and positive for WT TrkA protein)	Slide(2)	HCL038
	Slide(5)	HCL039
	Block	HCL040
Mismatch Repair Analyte Control[DR] (Four cores, intact expression for MLH1/PMS/MSH2/MSH6, loss of expression for MLH1/PMS2, loss of expression for MSH2, loss of expression for MSH2/MSH6)	Slide(2)	HCL041
	Slide(5)	HCL042
	Block	HCL043
MLH1/PMS2 Analyte Control (Two cores, one with MLH1 deletion and loss of expression of MLH1 and PMS2, one with intact expression for MLH1 and PMS2)	Slide(2)	HCL044
	Slide(5)	HCL045
	Block	HCL046
MSH2 Analyte Control (Two cores, one with loss of MSH2 expression, one with intact expression of MSH2)	Slide(2)	HCL047
	Slide(5)	HCL048
	Block	HCL049
MSH6 Analyte Control (Two cores, one with loss of MSH6 expression, one with intact expression of MSH6)	Slide(2)	HCL050
	Slide(5)	HCL051
	Block	HCL052

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Quality in Control

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