HistoCyte Laboratories

Quality in Control

HPV/p16 Analyte Controls

Product Introduction

Product Codes:

HCL001, HCL002, HCL003

HCL004, HCL005, HCL006

Issue 3. 220325

Contents

Product Name	Format	Code
HPV/p16 Receptor Analyte Control^{DR} (Four cores with a dynamic range of HPV gene copies)	Slide (2)	HCL001
	Slide (5)	HCL002
	Block	HCL003
HPV/p16 Receptor Analyte Control (Three cores with a standard range of HPV gene copies)	Slide (2)	HCL004
	Slide (5)	HCL005
	Block	HCL006

(For research use only)

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 low and intermediate expressing material that is consistent, one example being HER2 2+ tissue. Not only is it hard to find tissue in sufficient amounts, but biomarker expression can also vary throughout tissue, often due to a number of factors including but not limited to:

- Fixation
- Processing artefact
- Heterogeneity of the protein, see Figure 1 (taken from Nitta H *et al*[1])

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

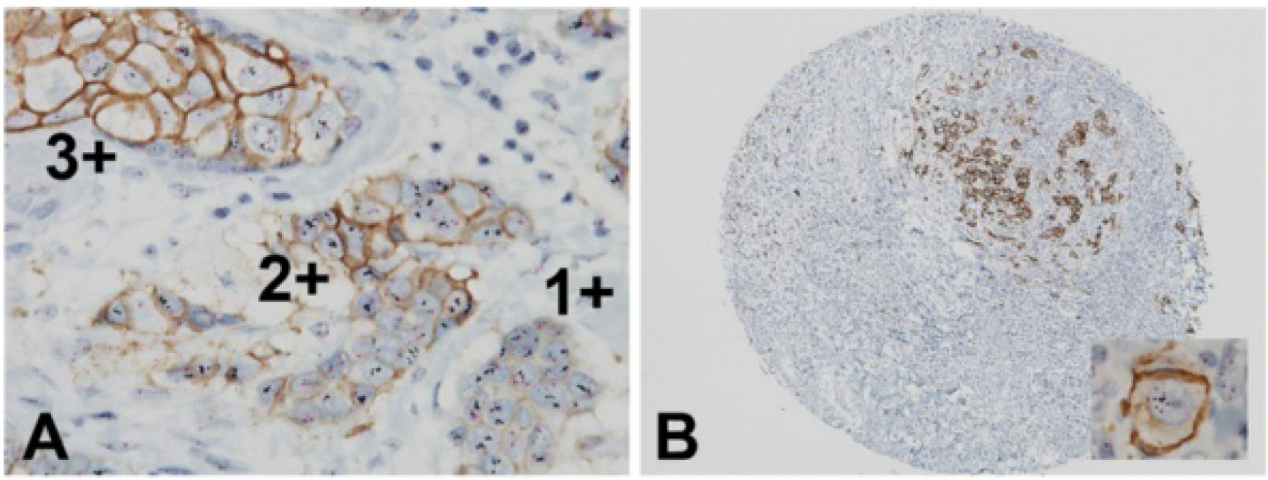


Figure 1. Results of HER2 gene-protein staining of FFPE breast cancer tissues exhibiting heterogeneity of HER2 positive tumor cell populations or isolated tumour cell populations. (A) The HER2 gene-protein assay demonstrated the heterogeneity of HER2 positive tumour cell populations in FFPE breast cancer 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].[6]

1. Nitta et al. Diagnostic Pathology 2012, 7:60

Cell lines

Cell lines are typically included in or with assays as pre-cut slides. These are not designed for use as same slide controls and pre-cut slides are not practical for day to day use in a high volume laboratory. They are 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 to provide standardized material there is room for improvement.

Our solution

HistoCyte Laboratories provide cell lines that 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, developed and manufactured to provide consistent results throughout the block. This is what differentiates them from tissue controls. It should be remembered that these still need validating in each laboratory that adopts them.

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.

Introduction to HPV

What is it?

Human Papillomavirus or HPV is a group of more than 150 related viruses, over 40 of which can be transmitted through direct skin-to-skin contact during vaginal, anal and oral sex. These sexually transmitted HPV subtypes fall into two categories:

- Low-Risk HPV, e.g. HPV 6 & 11, which do not cause cancer but are responsible for 90% of genital warts cases.
- High-Risk or Oncogenic HPV which can cause cancer. At least 15 high-risk HPV subtypes have been identified. Two of these, HPV 16 & 18, are responsible for the majority of HPV-related cancers.[1, 2]

Role of HPV in Cancer

High-risk HPV infection accounts for approximately 5% of all cancers globally.[3] That said, most HPV infections occur without symptoms and regress within 2 years without causing cancer. Some HPV infections, however, persist and can progress to cancer if left untreated. HPV, through expression of E6 and E7, has a negative impact by binding to the p53 and retinoblastoma tumour suppressor pathways, and as such, integration of the virus typically leads to an overexpression of $p16^{ink4A}$.[4]

Virtually all cases of cervical cancer are caused by HPV infection, with HPV 16 & 18 detected in 70%.[1,2] HPV 16 is responsible for around 85% of anal cancers and HPV 16 & 18 account for approximately 50% of vaginal, vulval and penile cancers.[5] Within the last 20 years, the incidence of HPV-associated oropharyngeal cancer has increased, particularly among men.

HPV 16 has been identified in around 50% of oropharyngeal cancers in the US.[6] Indeed it has been estimated that, by 2020, HPV will cause more oropharyngeal cancers than cervical cancers in the US.[7]

Detecting HPV& p16

HPV infection is detected using assays that detect viral DNA or RNA within the cell. p16 is commonly used as a surrogate marker of oncogenic HPV infection and can be demonstrated using immunohistochemistry. HPV DNA is most commonly assessed by PCR and *in-situ* hybridisation (ISH). Recently, more sensitive ISH assays, able to detect HPV mRNA E6 & E7, have come into routine use.

The **HPV/p16 Analyte Control** and **HPV/p16 Analyte Control^{DR}** have been developed for use as an analyte control for slide-based assays. They are available as pre-cut sections and blocks. The Dynamic Range (DR) format contains an extra cell line giving a Dynamic Range of HPV gene expression. In turn providing an extra degree of sensitivity compared to the standard HPV/p16 Analyte Control. The viral genomes are incorporated into the host DNA but there is no virus associated protein as these genes are not transcribed. Therefore, for example, antibodies to HPV capsid proteins will not work in techniques such as immunohistochemistry.

1) Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S. Human papillomavirus and cervical cancer. *Lancet* 2007; 370(9590):890–907.
2) Muñoz N, Bosch FX, Castellsagué X, et al. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. *International Journal of Cancer* 2004; 111(2):278–285.
3) Parkin DM. The global health burden of infection-associated cancers in the year 2002. *International Journal of Cancer* 2006; 118(12):3030–3044.
4) Kong et al. The relationship between human papillomavirus status and other molecular prognostic markers in head and neck squamous cell carcinomas. *Int J Radiat Oncol Biol Phys*. 2009; 74(2): 553-561.
5) Watson M, Saraiya M, Ahmed F, et al. Using population-based cancer registry data to assess the burden of human papillomavirus-associated cancers in the United States: overview of methods. *Cancer* 2008; 113(10 Suppl):2841–2854.
6) Jayaprakash V, Reid M, Hatton E, et al. Human papillomavirus types 16 and 18 in epithelial dysplasia of oral cavity and oropharynx: a meta-analysis, 1985–2010. *Oral Oncology* 2011; 47(11):1048–1054.
7) Chaturvedi AK, Engels EA, Pfeiffer RM, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *Journal of Clinical Oncology* 2011; 29(32):4294–4301.

HPV/p16 Analyte Control^{DR}

The **HPV/p16 Analyte Control^{DR}** is sold in two formats: as pre-prepared slides (Figure 2) or as a cell microarray (CMA) paraffin wax block (Figure 3).

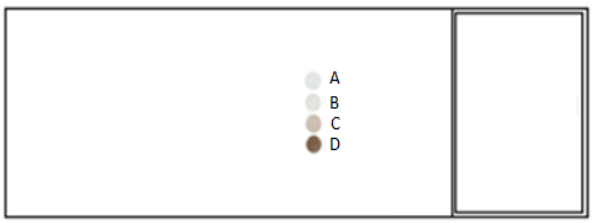


Figure 2: Cell Line Control Slide

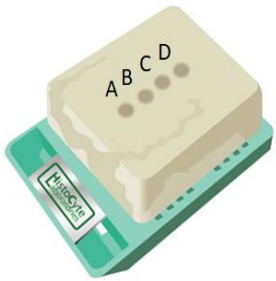


Figure 3: CMA block

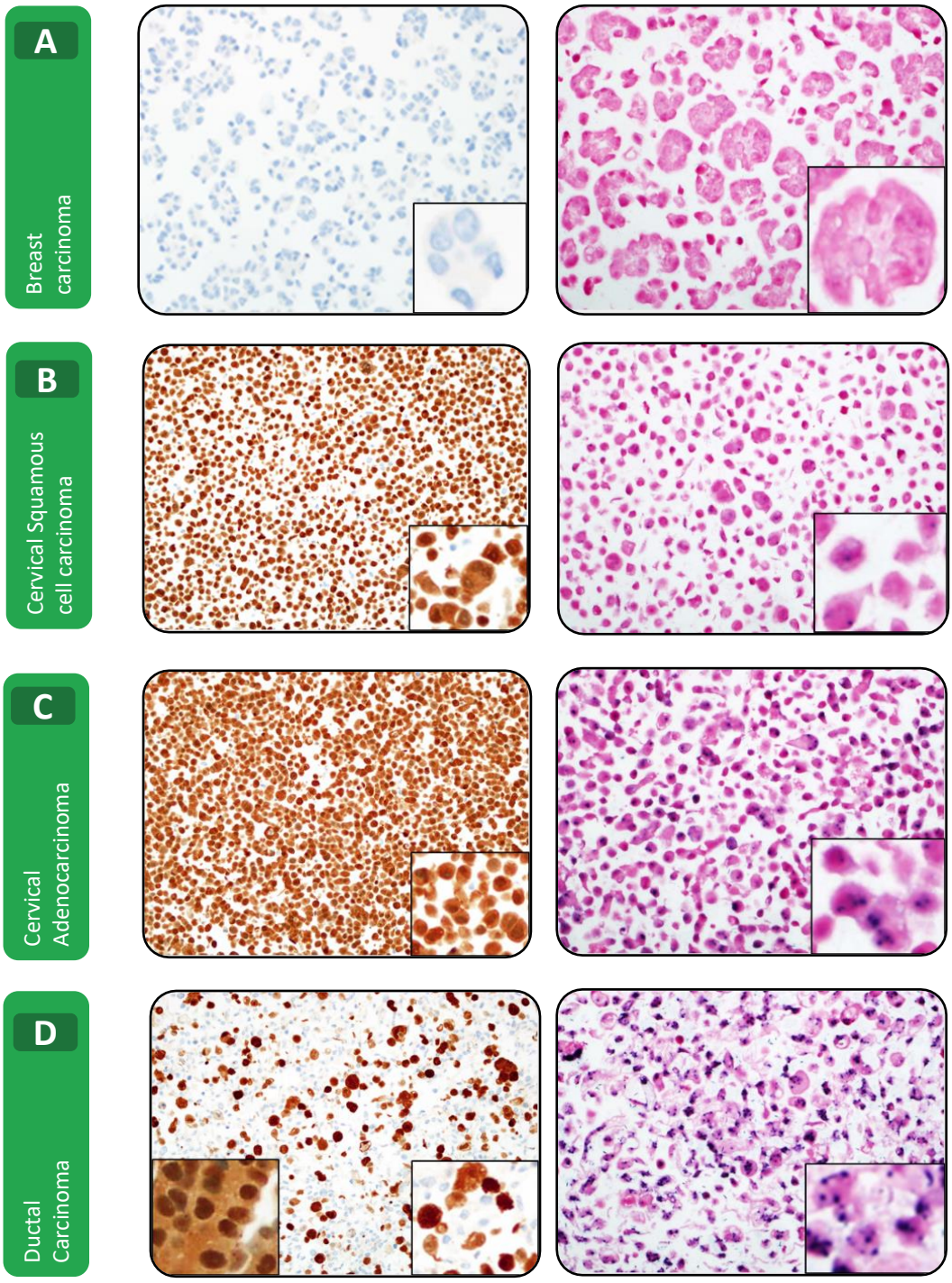
Our CMA block provides the most cost effective solution for clinical histology laboratories and other high volume centers. They have been purposely designed to fit seamlessly into the work flow of the laboratory.

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 4 cell lines for p16 IHC, HPV ISH and E6/E7 ISH are shown below. Cell B contains low numbers of HPV gene copies providing greater sensitivity over the standard control HPV/p16 Analyte Control.

	Cell Lines	HPV Gene Status	p16 Expression
A	Breast Adenocarcinoma	Negative	Negative
B	Cervical Squamous Cell Carcinoma	Low (1-2) HPV gene copy[8]	High
C	Cervical Adenocarcinoma	Medium HPV gene copy	High
D	Epidermoid Carcinoma	High HPV gene copy	High (heterogeneous)

HPV/p16 Analyte Control^{DR} Expected Results

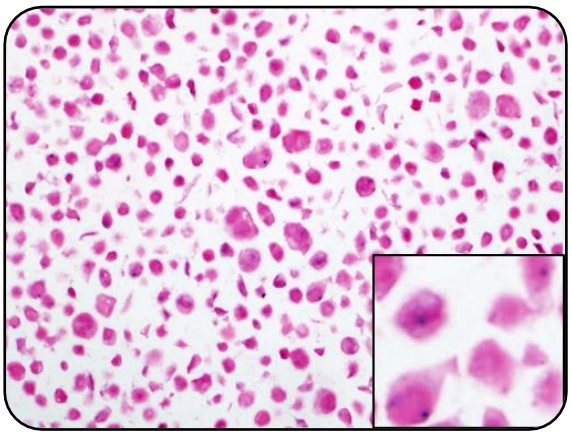


Roche/Ventana	Roche/Ventana
CONFIRM *CINtec® p16 Histology, E6H4™* (Product Code: 805-4713)	INFORM® HPV III Family probe (Product Code: 800-4295)

Staining Observations

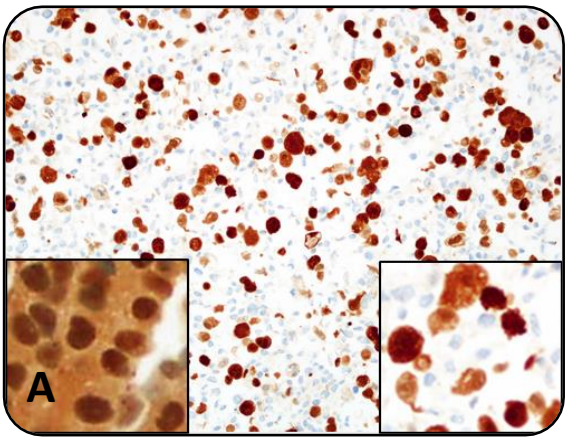
Cell B – HPV ISH

1-2 gene copies are observed in those cells that are positive. It is recommended that this cell line is reviewed using a x40 objective.



Cell D – p16 IHC

30-50% of the cells demonstrate intense nuclear and cytoplasmic staining. Cell in core D are typically more homogeneously positive for p16 by IHC (see inset A). HistoCyte Laboratories Ltd have manipulated the cells to provide a heterogeneous analyte control. 30-50% of the cells should stain compared to no staining in the negative cell line A and 100% of cells staining in the high expressers (Cell lines B and C).



HPV/p16 Analyte Control

The **HPV/p16 Analyte Control** is sold in two formats: as pre-prepared slides (Figure 4) or as a cell microarray (CMA) paraffin wax block (Figure 5).

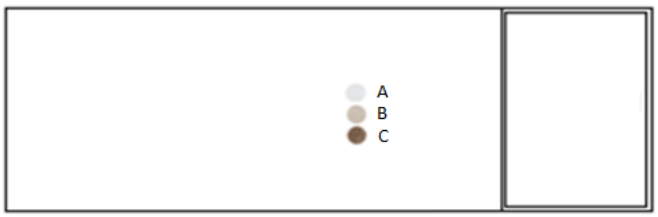


Figure 4. Cell Line Control Slide

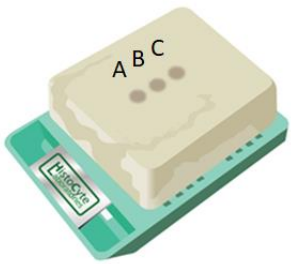


Figure 5. CMA/Block

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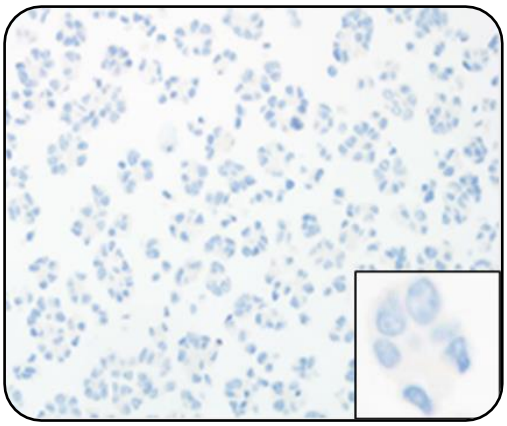
Our pre-prepared slides offer a ready-to-go alternative that saves time in preparation. These are ideal for one-off assessments, research laboratories and preliminary product trials.

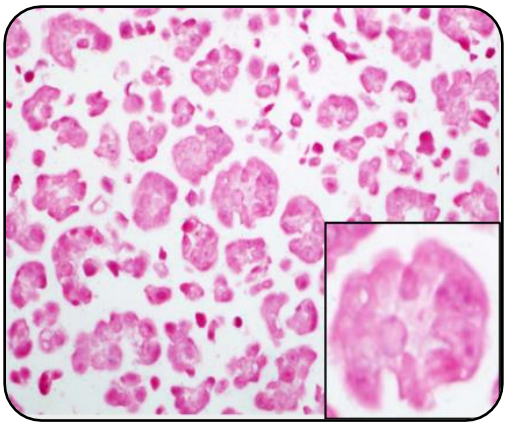
The expression patterns of the 3 cell lines for p16 IHC, HPV ISH and E6/E7 ISH are shown below. This version of the product is more cost effective but has less sensitivity as there is no low HPV gene expressing cell line.

	Cell Lines	HPV Gene Status	p16 Expression
A	Breast Adenocarcinoma	Negative	Negative
B	Cervical Adenocarcinoma	Medium HPV gene copy	High
C	Epidermoid Carcinoma	High HPV gene copy	High (heterogeneous)

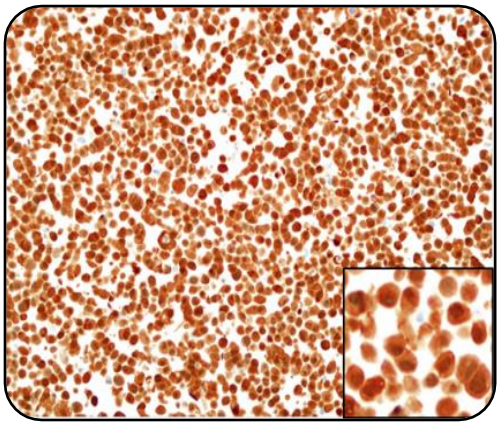
HPV/p16 Analyte Control^{DR} IHC

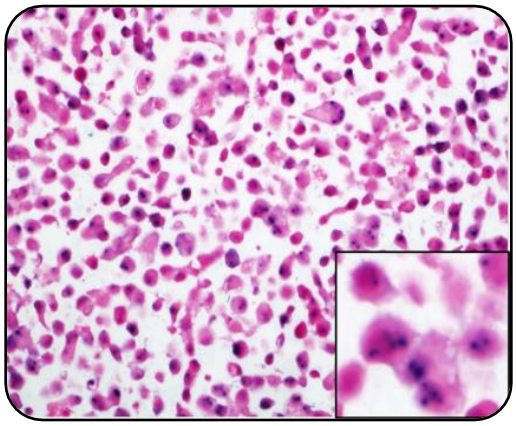
A Breast carcinoma



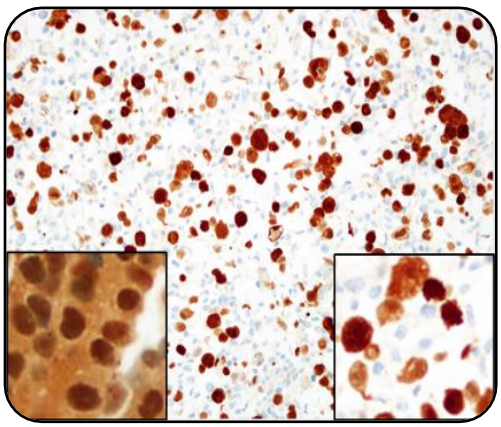


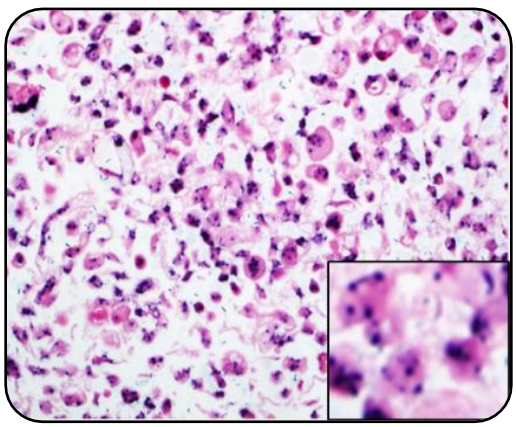
B Cervical Adenocarcinoma





C Ductal Carcinoma





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Staining Observations

Cell C – p16 IHC

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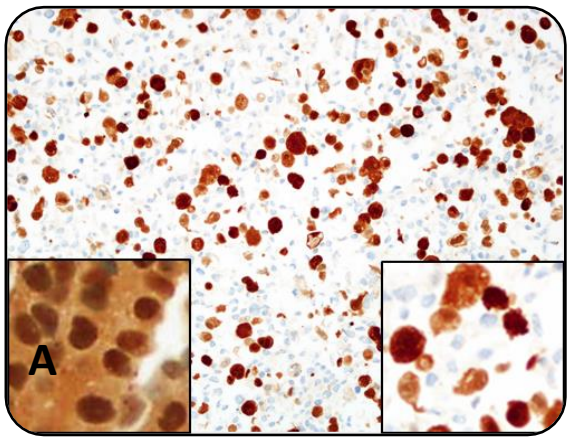
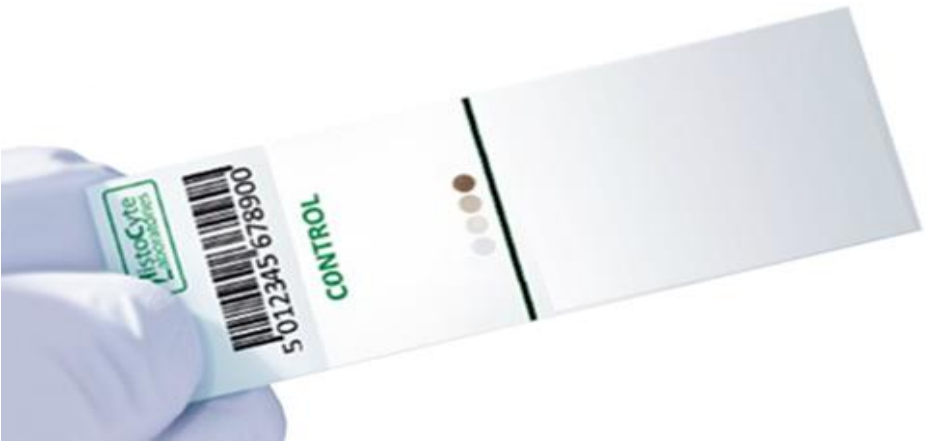


HistoCyte Laboratories

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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