HistoCyte Laboratories

Quality in Control

HPV/p16 Analyte Controls

Product Introduction

Product Codes:

HCL001, HCL002, HCL003

HCL004, HCL005, HCL006

Issue 4. 110123

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

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).

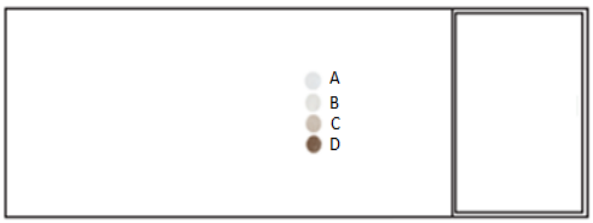


Figure 2: Cell Line Control Slide

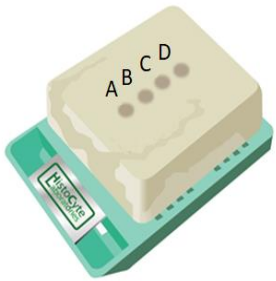


Figure 3: CMA block

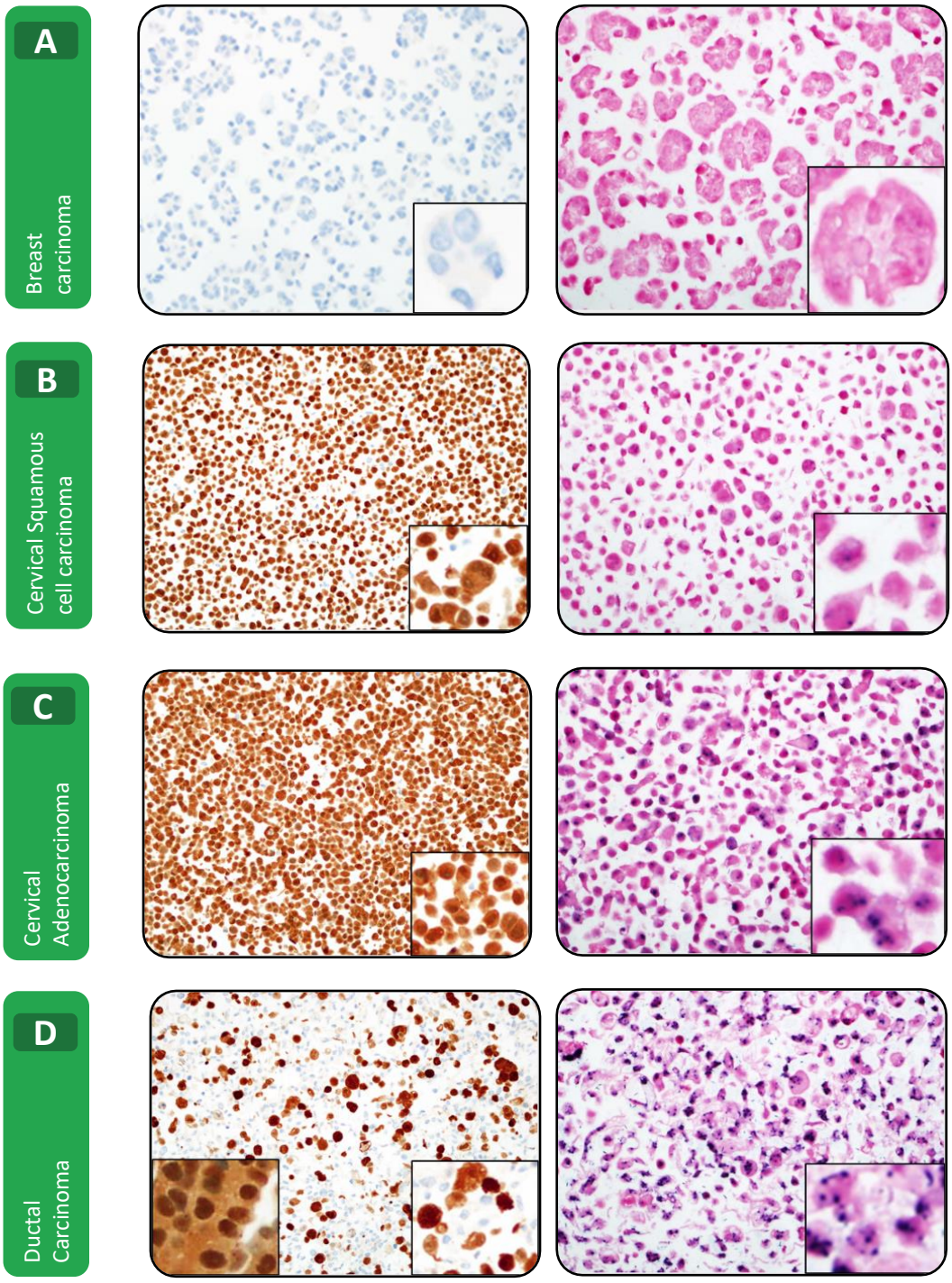
Our CMA block provides the most cost effective solution for clinical histology laboratories and other high volume centers. They have been purposely designed to fit seamlessly into the work flow of the laboratory.

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

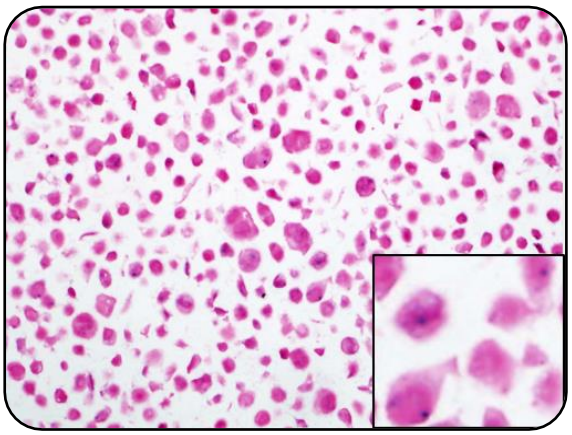


Roche/Ventana	Roche/Ventana
CONFIRM *CINtec® p16 Histology, E6H4™* (Product Code: 805-4713)	INFORM® HPV III Family probe (Product Code: 800-4295)

Staining Observations

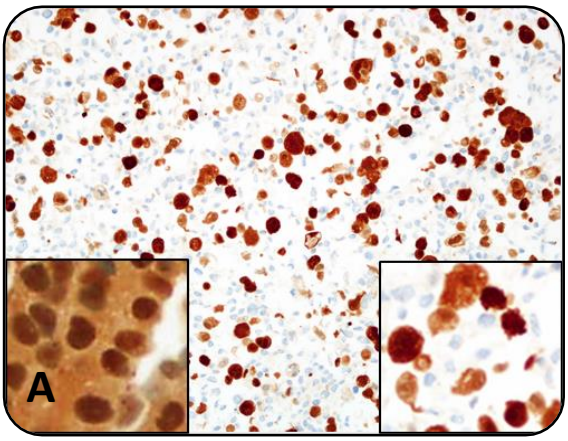
Cell B – HPV ISH

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).

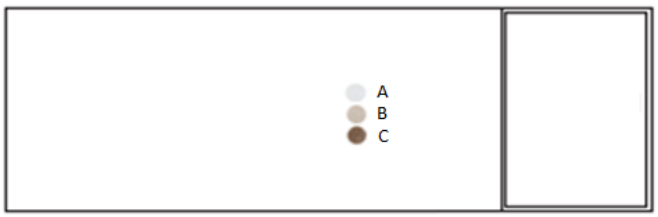


Figure 4. Cell Line Control Slide

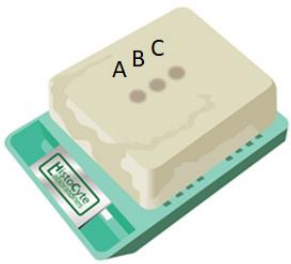


Figure 5. CMA/Block

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.

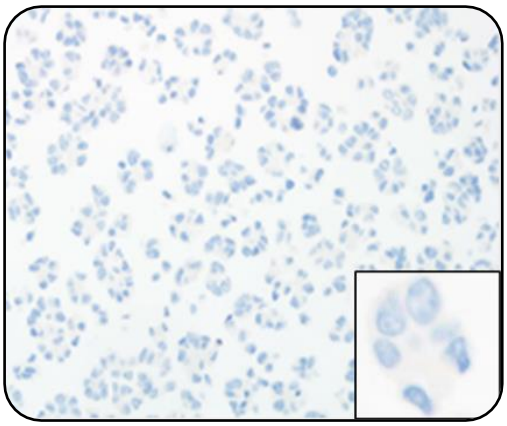
Our pre-prepared slides offer a ready-to-go alternative that saves time in preparation. These are ideal for one-off assessments, research laboratories and preliminary product trials.

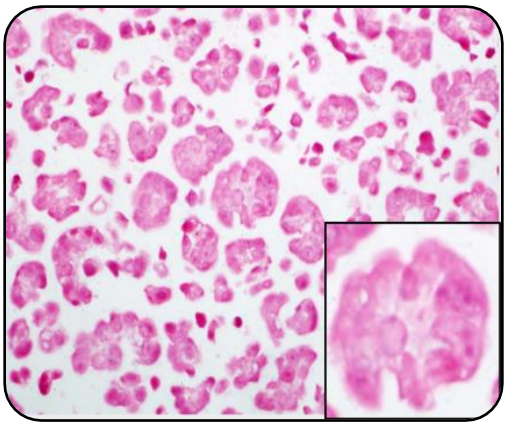
The expression patterns of the 3 cell lines for p16 IHC, HPV ISH and E6/E7 ISH are shown below. This version of the product is more cost effective but has less sensitivity as there is no low HPV gene expressing cell line.

	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)

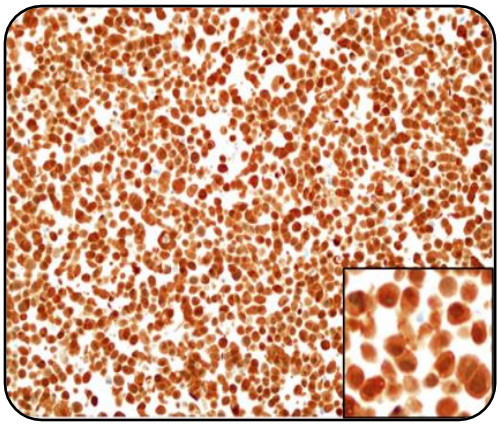
HPV/p16 Analyte Control^{DR} IHC

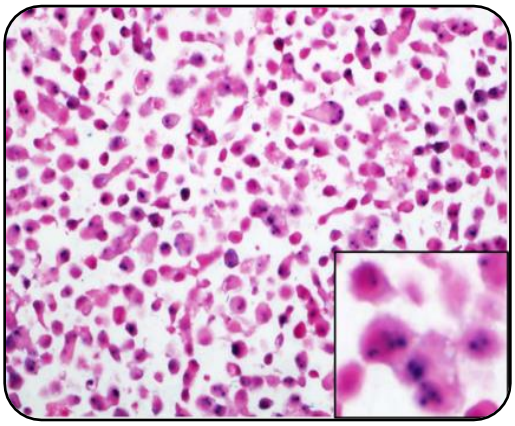
A Breast carcinoma



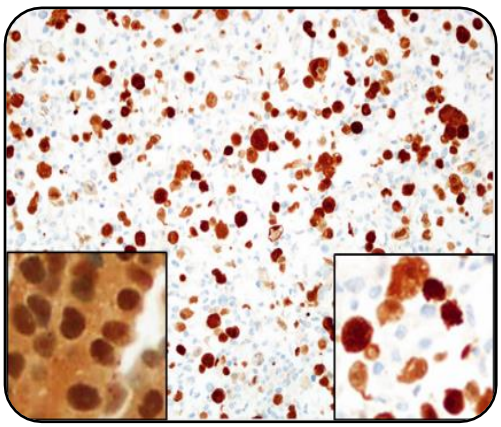


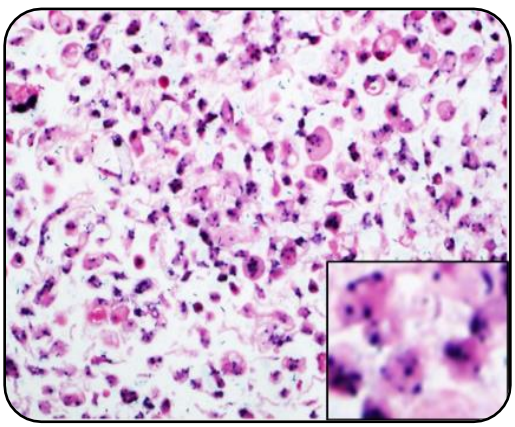
B Cervical Adenocarcinoma





C Ductal Carcinoma





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