Interpretation Guide

Product Name: HPV/p16 Analyte Control

Product Code: HCL004, HCL005 and HCL006

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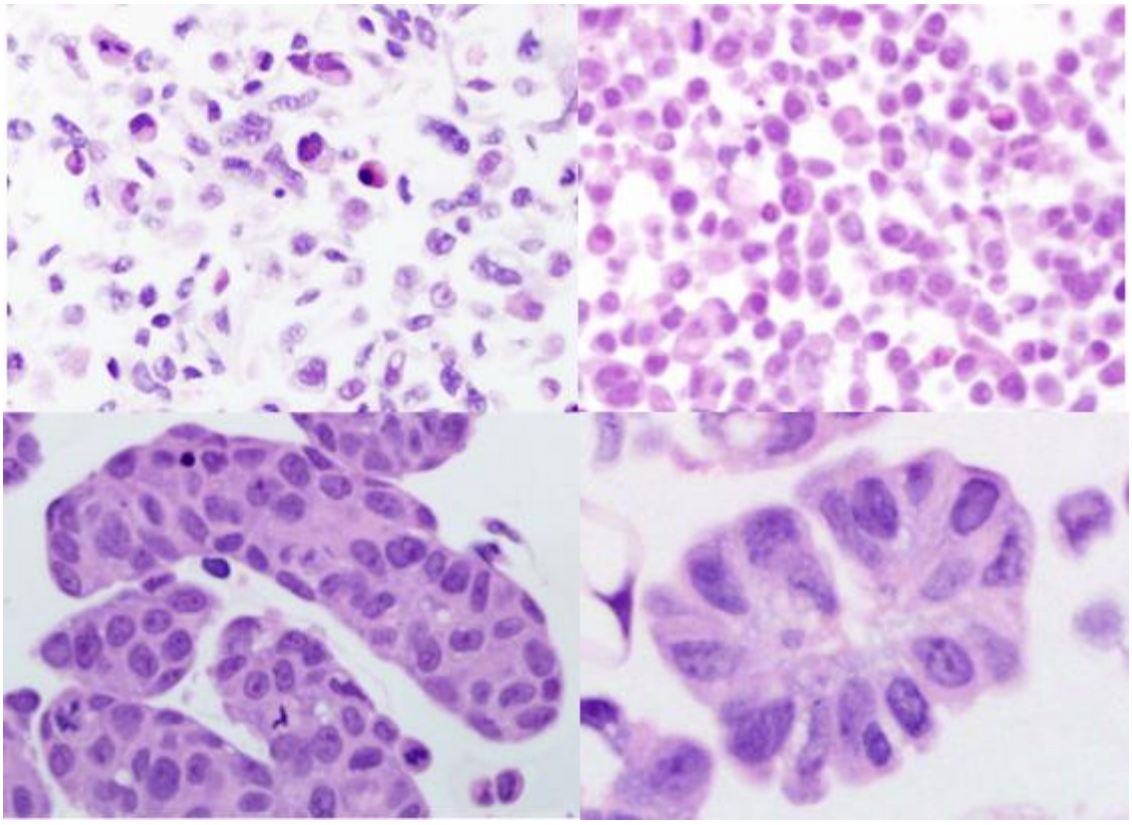
HistoCyte Laboratories Ltd is based in the heart of the Newcastle University campus. Started in 2014 by scientists with a combined experience of over 50 years in the development of reagents for immunohistochemistry and in-situ hybridization. Collaborating with pathologists locally and globally, HistoCyte 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 HPV?

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]

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]

3. 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.

Pre-cut section and block formats of **HPV/p16 Analyte Control** have been developed for use as an analyte control for slide-based assays.

4. Cell Line Controls

This product is sold in two formats. Pre-prepared slides: ***HCL004 and HCL005***, as in figure 1 below.

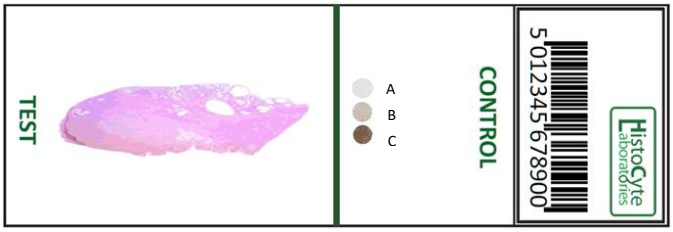


Figure 1: Pre-cut Cell Line Control Slide

Or in a cell microarray (CMA) paraffin wax block: ***HCL006***, as illustrated below.

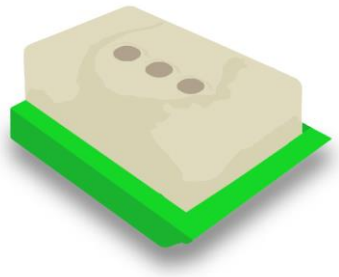


Figure 2: CMA block

Both formats have their merit and depending on the needs of the laboratory, the slides cater to ease of use and time saving. However, the blocks can reduce running costs 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 optimisation and assay 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, then the assay has worked and that any staining present within the sample is genuine.

The expression patterns of the 3 cell lines contained within **HPV/p16 Analyte Control** are shown in the table below:

Cell Lines			HPV Gene Status	E6/E7 mRNA	p16 Expression
A	MCF-7	Human Breast Adenocarcinoma	Negative	Negative	Negative
B	HeLa	Human Cervical Adenocarcinoma	Medium HPV gene copy	High	High
C	CaSki	Human Epidermoid Carcinoma	High HPV gene copy	High	High (heterogeneous)

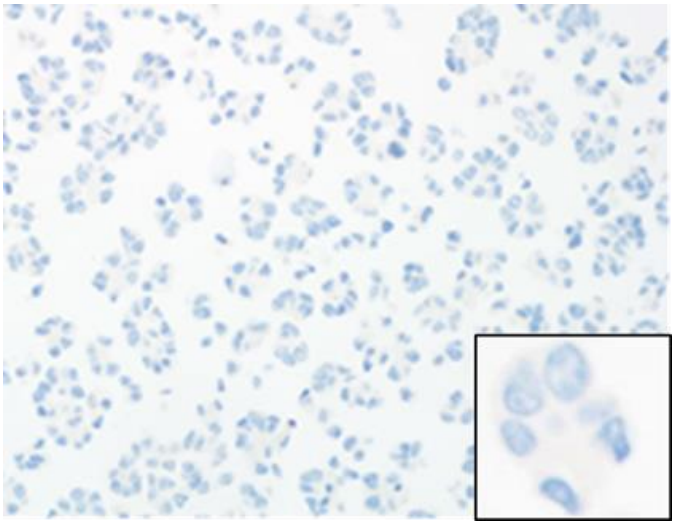
If greater sensitivity is required in the control the **HPV/p16 Analyte Control^{DR}** contains a low HPV gene expressing cell line and may well suit your needs.

5. Expected Staining Results

The following section gives micrographs of the expected results obtained with each of the cells with p16 immunohistochemistry, HPV DNA in-situ hybridisation and E6/E7 mRNA in-situ hybridisation. For more information please email info@histocyte.com

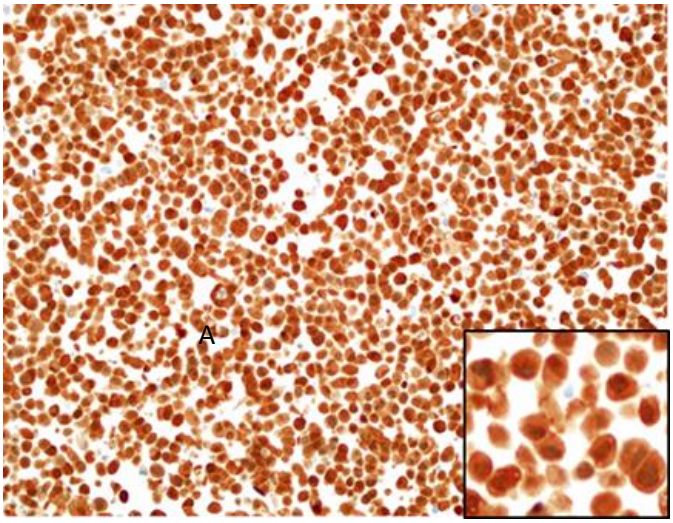
5.1 p16 Immunohistochemistry

MCF-7



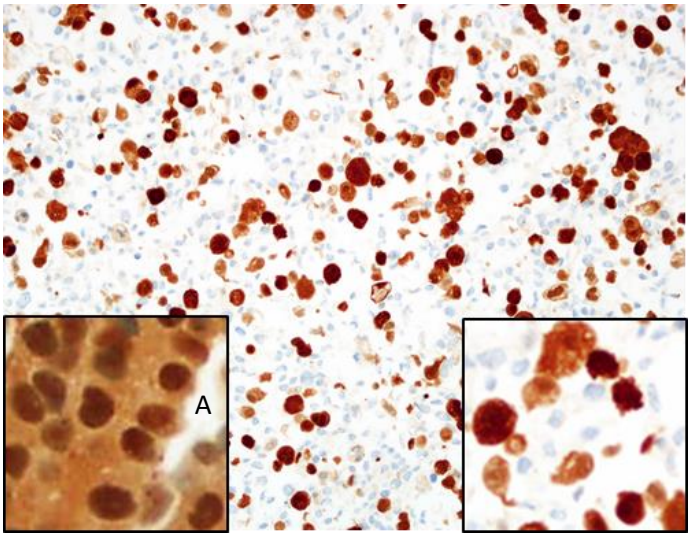
No staining seen in the MCF-7 cell line.

HeLa



>95% of cells demonstrate intense nuclear and cytoplasmic staining.

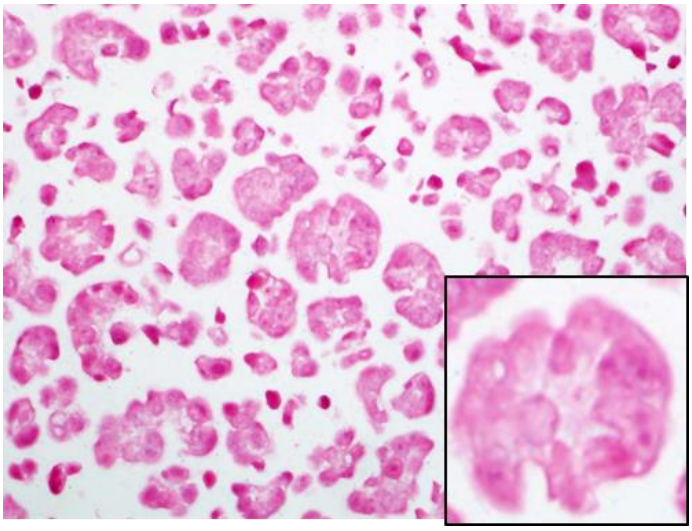
CaSki



30-50% of the cells demonstrate intense nuclear and cytoplasmic staining. CaSki cells 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 (MCF-7) cell line and 100% of cells staining in the high expresser (SiHa and HeLa) cell lines.

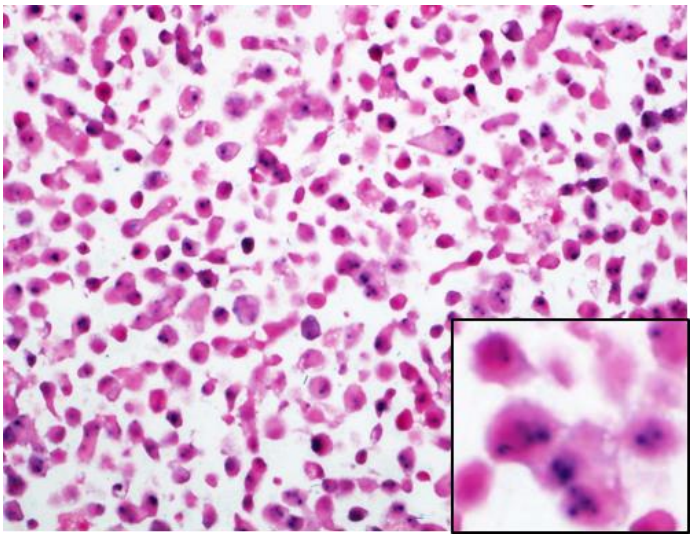
5.2 HPV DNA in-situ hybridization

MCF-7



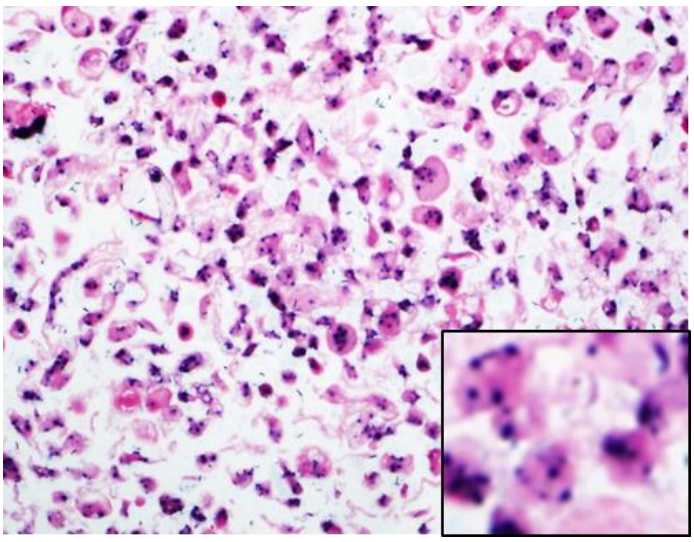
No staining seen in the MCF-7 cell line

HeLa



Majority of cells demonstrate moderate punctate nuclear staining. Multiple gene copies create intense clusters in many of the cells.

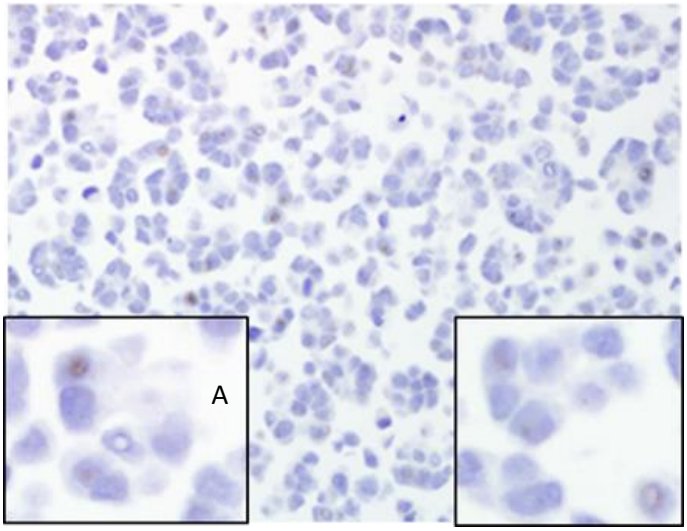
CaSki



The cells demonstrate a variety of staining patterns, from single punctate nuclear staining to multiple foci of signals in the nucleus.

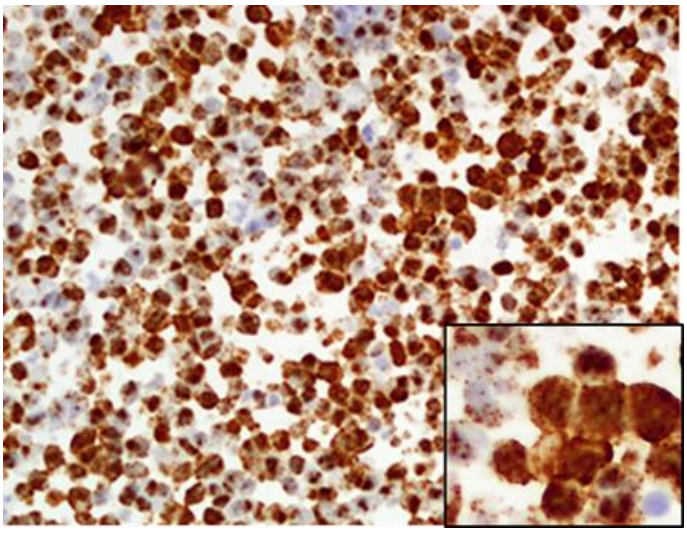
5.3 HPV E6/E7 mRNA in-situ hybridization

MCF-7



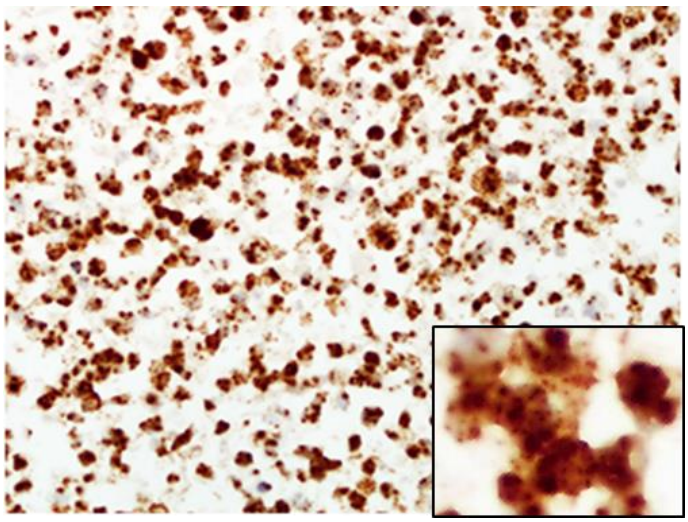
The cells are negative however, there is occasional perinuclear staining (see inset A). This is an artefact of the detection system and noted on the negative control probe DapB (image not included).

HeLa



The majority of cells demonstrate intense punctate staining. Typically cytoplasmic but in some cells there are signals in the nuclei too. This is likely due to mRNA being transcribed but also, with some cells, the plane in which the cells have been sectioned.

CaSki



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6. References

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.
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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.

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