HistoCyte Laboratories

Quality in Control

HER2 Analyte Control^{DR}

Product Introduction

Product Codes: HCL026, HCL027 and HCL028

Issue 2. 180307

Contents

HER2 Analyte Control^{DR} is available as pre-cut slides (2 or 5 slide options) and cell microarray blocks*.

Product Name	Format	Code
HER2 Analyte Control^{DR}	Slide (2)	HCL026
	Slide (5)	HCL027
	Block	HCL028

*For research use only

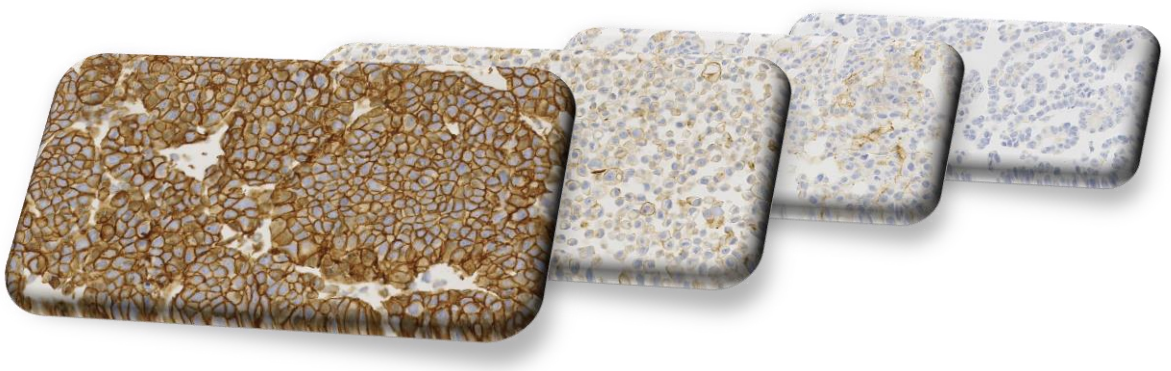
Introduction to HER2

What is it?

Human Epidermal growth factor Receptor 2 (HER2) also known as ERBB2, c-erbB2 is a tyrosine kinase receptor. It has no known specific ligand but does dimerise with other receptors of the same family (HER1 or EFGR through to HER4) as well as homo-dimerise, in order to facilitate signalling via these receptors.[1]

Utility

Over expression is associated with a number of cancers such as breast and gastric and demonstration of this drives therapeutic decisions with a variety of drugs, primarily trastuzumab (Herceptin®). Pathological samples are assessed by immunohistochemistry (IHC) and/or fluorescence in situ hybridisation (FISH). IHC is scored according to the proportion of tumour cells that have complete, strong membrane staining. FISH determines whether the tumor cells have amplified HER2 gene present. As of 2013 the guidelines for HER2 assessment were updated by the American Society of Clinical Oncology and College of American Pathologists.[2] These guidelines were subsequently adopted elsewhere for example in the UK as described by Rakha et al.[3] Figure 1. is an overview of the scoring algorithm for both IHC and ISH.



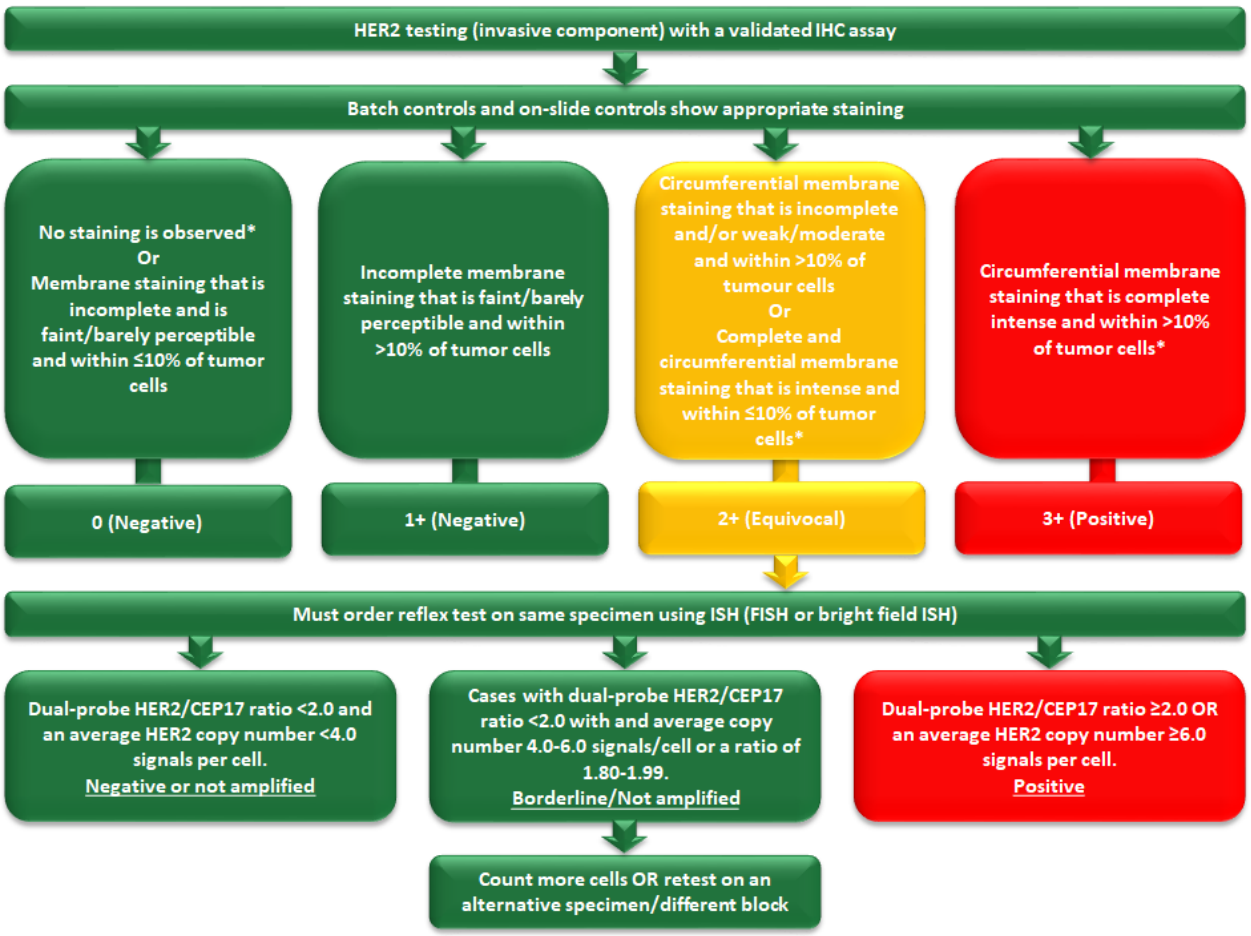


Figure 1. Recommended HER2 scoring algorithm for immunohistochemistry (IHC) and in situ hybridisation (ISH). Adapted from Wolf[1] et al and Rakha et al[2] *Membrane staining must be intense and uniform and resemble chicken-wire. Ignore incomplete or pale membrane staining in the percentage estimation.

HER2 immunohistochemistry

The tests

HercepTest™ was the first companion diagnostic for HER2 from Dako (Agilent) launched in 1998. Over the following 10 years Ventana Medical Systems (Roche) and Leica Biosystems launched their own assays.

While there are many antibodies available to the HER2 protein that laboratories can create laboratory developed tests (LDT) from, there are still only the following three standardized automated assays available on the market:

1. Agilent's Dako HercepTest™. This contains a rabbit polyclonal antibody. This was the first HER2 companion diagnostic.
2. Leica Biosystems, Bond Oracle HER2 system. This has the CB11 mouse monoclonal antibody.
3. Roche, Ventana PATHWAY anti-HER2. This contains the rabbit monoclonal antibody 4B5.

External Quality Assurance

Initially due to cost many laboratories used LDTs. With an increase in full automation of assays and reduced cost per test there has been wider adoption of these standardized assays.

Another driver has been external quality assurance (EQA) schemes or proficiency testing (PT) that have shown these assays typically perform better than LDTs. As laboratories switched to the standardized assays the overall quality of assessments performed in laboratories has improved.[4] There are still laboratories that use LDTs but as the EQA schemes results show the highest pass rates are typically with the standardized assays, see figure 2, below.[5]

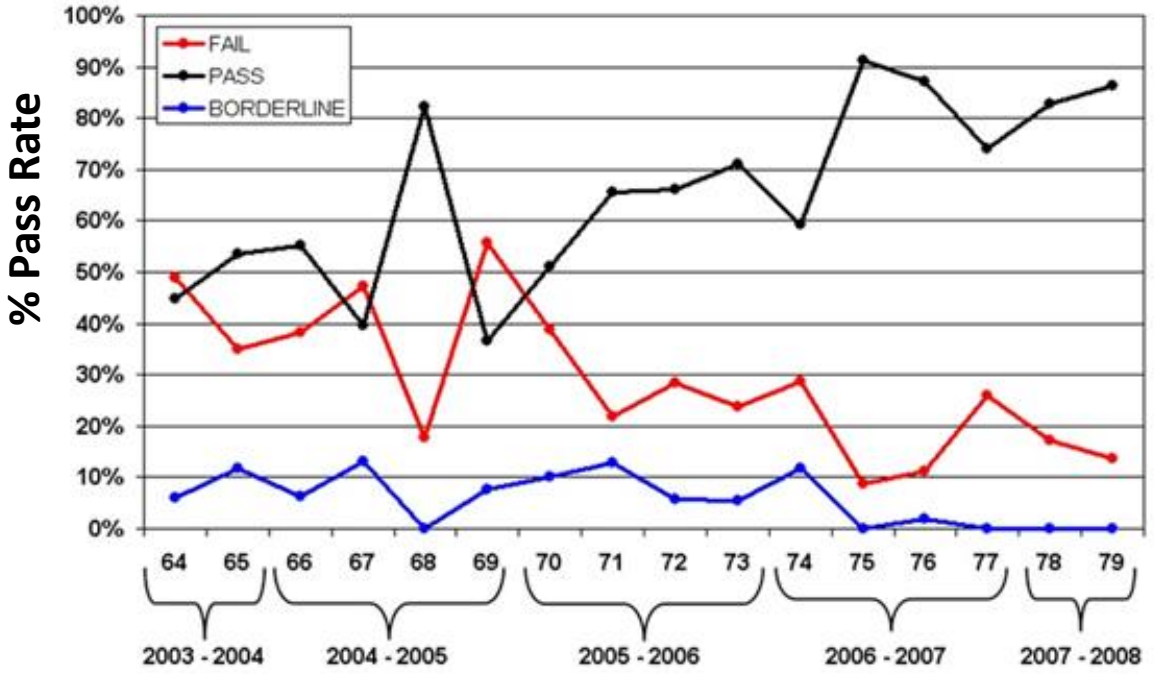


Figure 2. Overall pass rate improvement as subsequent United Kingdom National External Quality Assurance Scheme for Immunocytochemistry and In Situ Hybridization (UKNEQAS-ICC&ISH). This has been attributed in part to standardized assays being adopted over LDTs.

Quality Control

One of the requirements of quality standardization is the appropriate use of controls. These need to be robust enough for IHC and 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.

Cell Lines as Controls

The issue with tissue

Laboratories often struggle for HER2 2+ and sometimes HER2 1+ tissues. 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*[6])

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

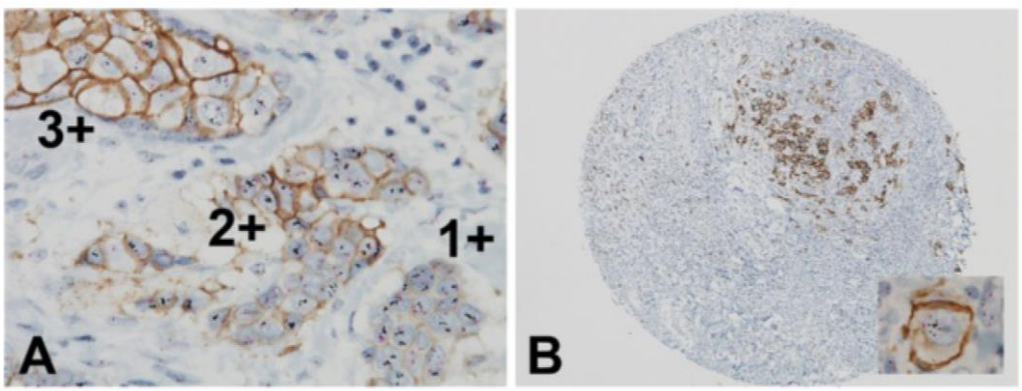


Figure 3. Results of HER2 gene-protein staining of FFPE breast cancer tissues exhibiting heterogeneity of HER2 positive tumor cell populations or isolated tumour cell populations. (A) The HER2 gene-protein assay demonstrated the heterogeneity of HER2 positive tumour cell populations in FFPE breast cancer 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]

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.

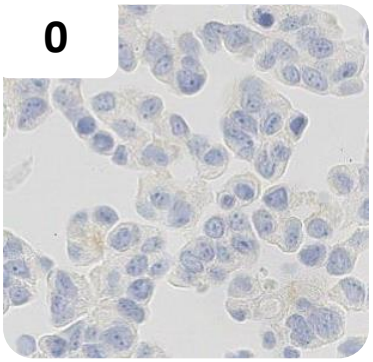
Tissue is still important

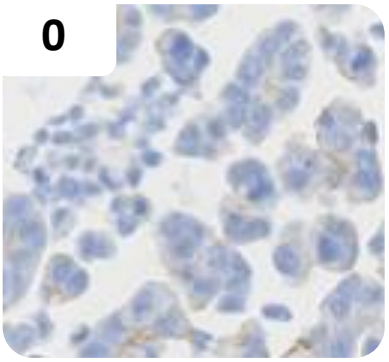
It is important to remember that these are a quality control material designed only to demonstrate that the assay has worked consistently. They reduce the burden on a laboratory to identify and obtain suitable materials for use as a same slide control. This means tissue can be preserved for other uses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

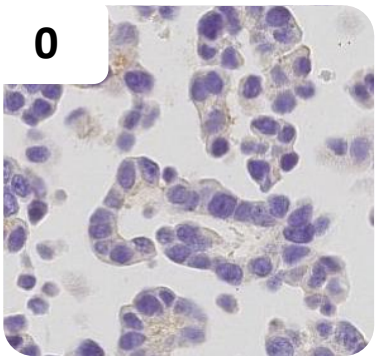
1. Brennan PJ, Oncogene 19, 6093-6101, 11 Dec 2000
2. Wolf, AC et al. Arch Pathol Lab Med. 2014;138:241–256;
3. Rakha, EA et al. J Clin Pathol doi:10.1136/jclinpath-2014-202571
4. Vyberg, M. & Nielsen, S. Virchows Arch (2016) 468: 19
5. Chapter 6. Standard Reference Material: Cell Lines Development and Use of Reference Cell Lines as Standards for External Quality Assurance of HER2 IHC and ISH Testing. In Taylor C, Shi S (eds.) : Wiley-Blackwell; 2010. p101-122.
6. Nitta et al. Diagnostic Pathology 2012, 7:60

HER2 Analyte Control^{DR} IHC

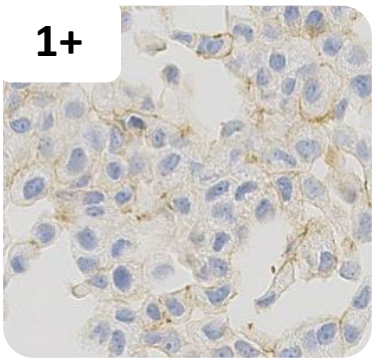
A Breast adenocarcinoma

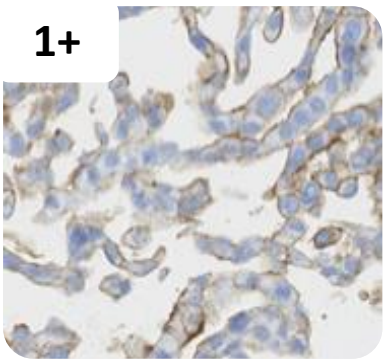


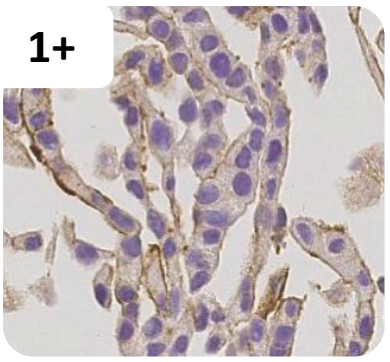




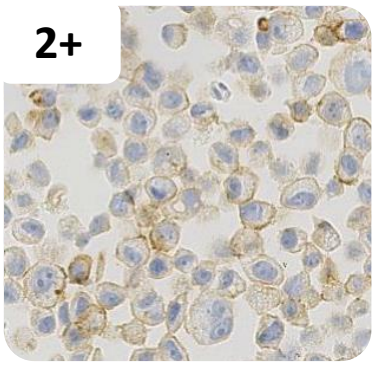
B Breast adenocarcinoma

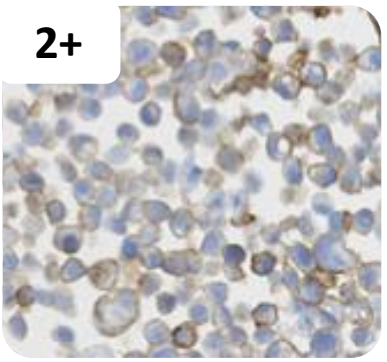


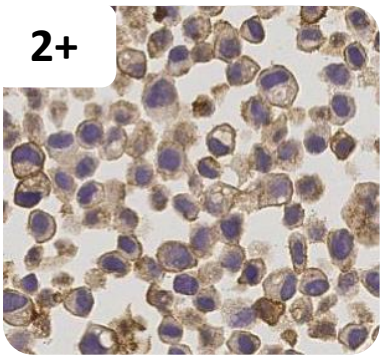




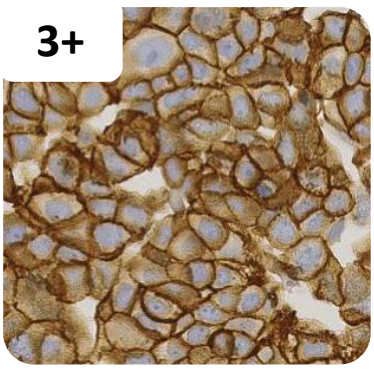
C Gastric adenocarcinoma

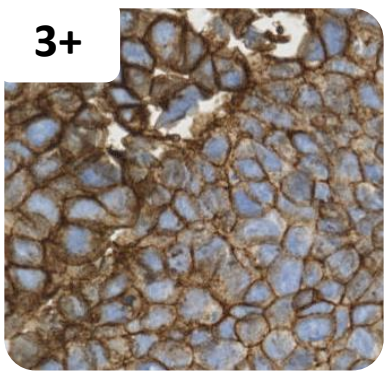


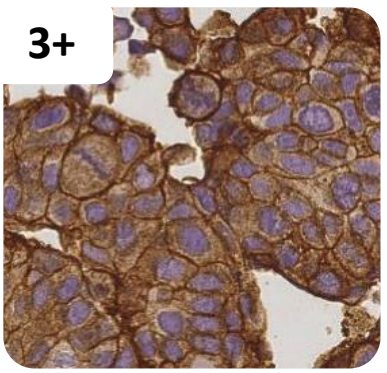




D Breast adenocarcinoma

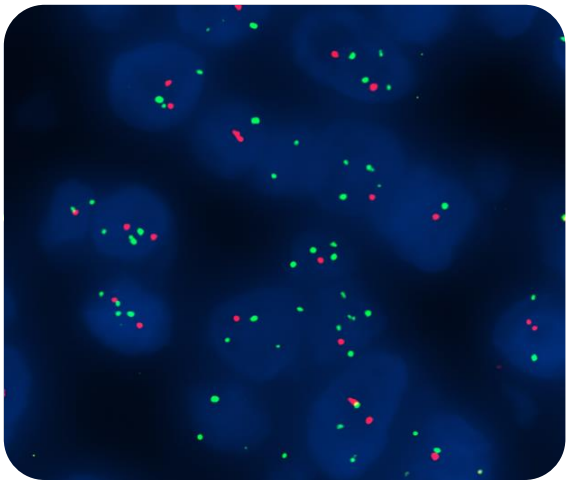






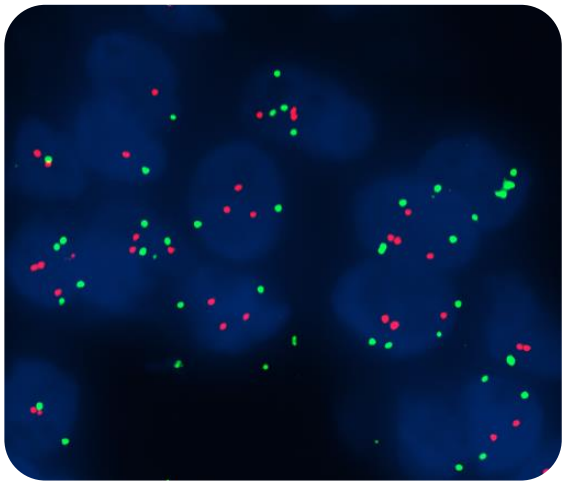
Roche/Ventana	Agilent	Leica Biosystems
PATHWAY anti-HER-2/neu (4B5) Rabbit Monoclonal Primary Antibody	**Dako HercepTest™ Rabbit polyclonal Antibody**	**Bond™ Oracle™ HER2 IHC System. CB11 Mouse Monoclonal Primary Antibody**

HER2 Analyte Control^{DR} FISH



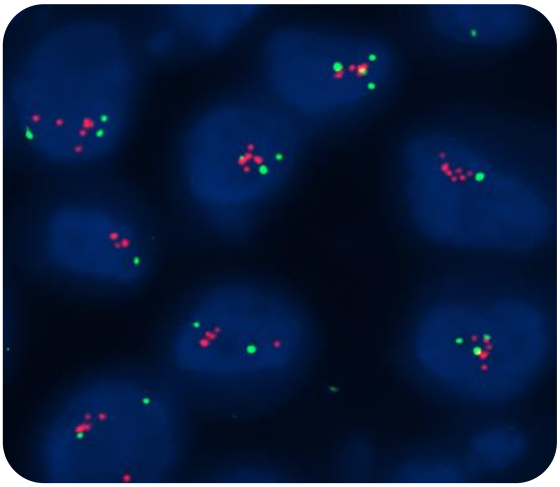
A Breast adenocarcinoma

Non-amplified



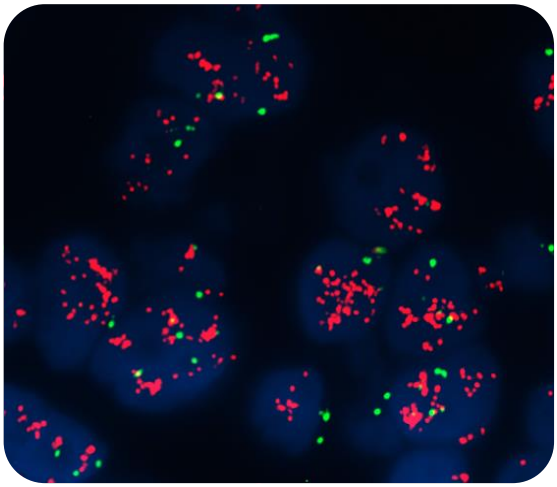
B Breast adenocarcinoma

Non-amplified



C Gastric adenocarcinoma

Equivocal/Borderline



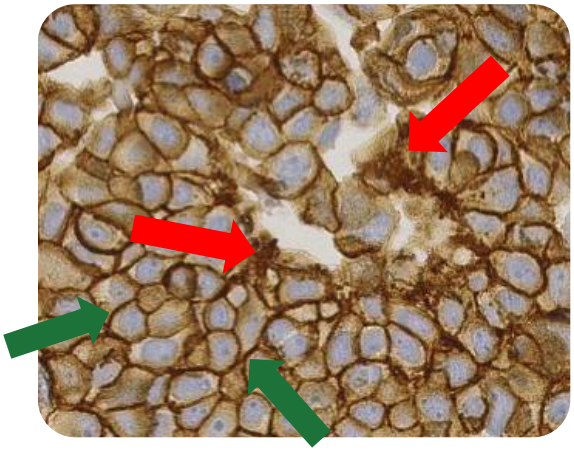
D Breast adenocarcinoma

Amplified

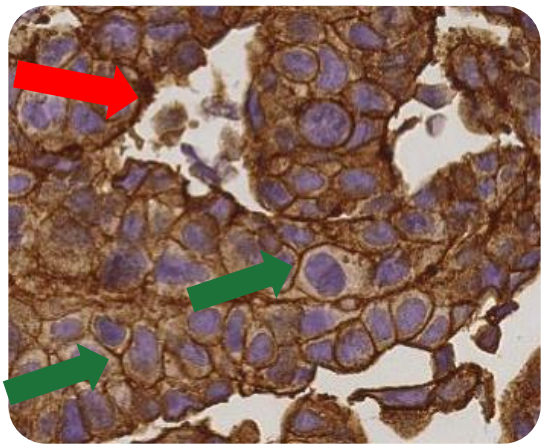
Abbott PathVysion® Vysis HER2 Gene Probe Kit

3+ Cell line staining

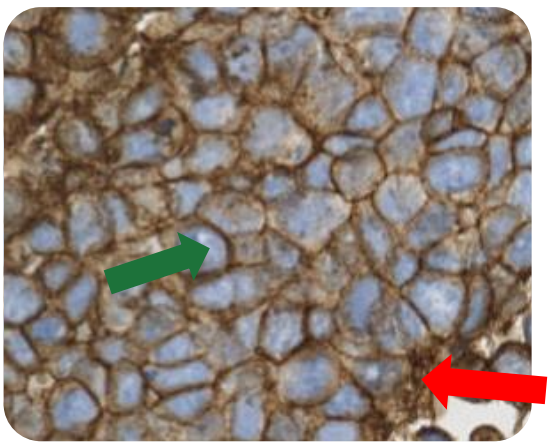
Roche/Ventana



Leica Biosystems



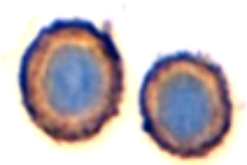
Agilent



HER2 Guidelines 3+

Circumferential membrane staining that is complete intense and within >10% of tumor cells*

Due to the way the cells are processed by HistoCyte the cells replicated the "chicken wire" effect seen in tissue. They would otherwise look sparse and more typical of cell preparations:

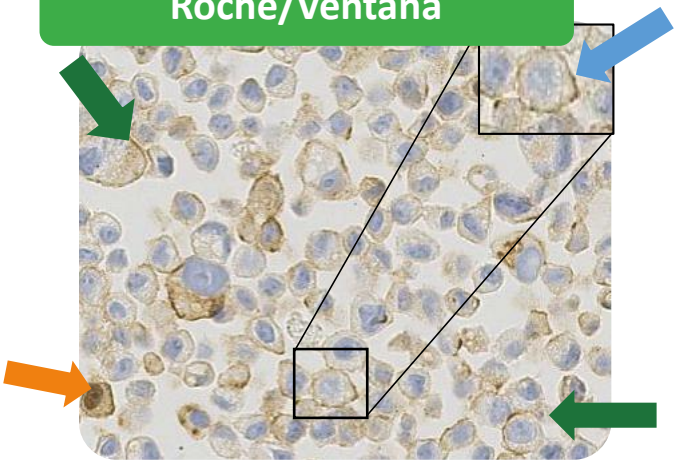


The periphery of the cell formations or "clumps" should not be scored (red arrows), it is within the clumps between adjoining cells where there is clear cell to cell contact (green arrows) that membrane should be scored.

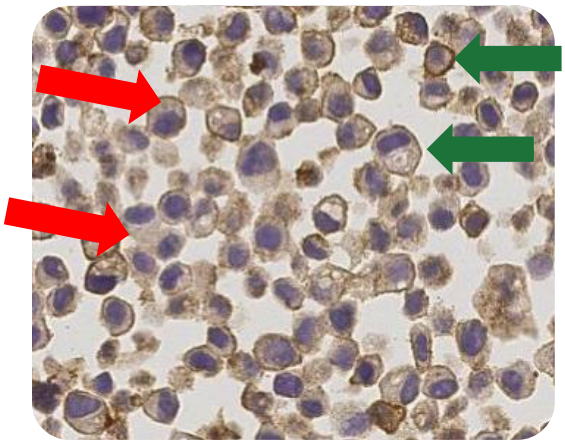
In each case we have intense circumferential membrane staining.

2+ Cell line staining

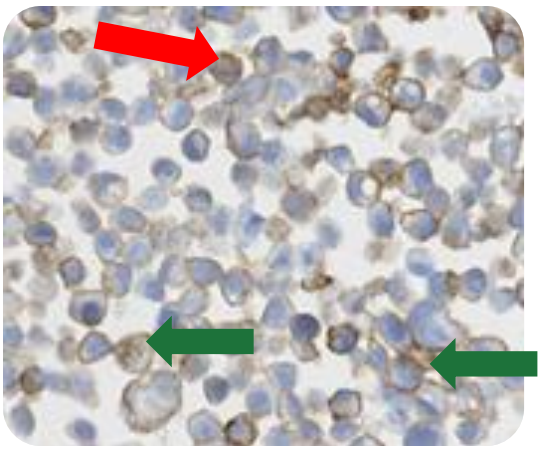
Roche/Ventana



Leica Biosystems



Agilent



HER2 Guidelines 2+

(a) Circumferential membrane staining that is incomplete and/or weak/moderate and within >10% of tumor cells

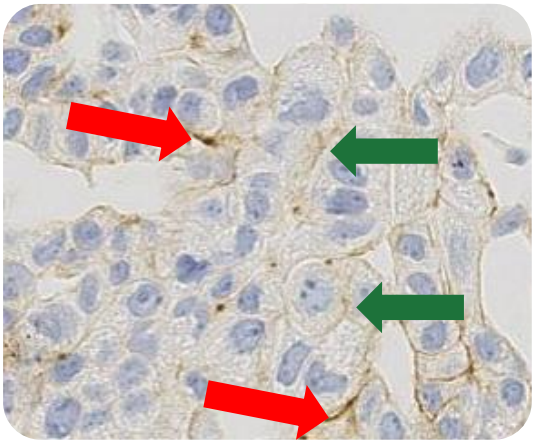
OR

(b) Complete and circumferential membrane staining that is intense and within ≤10% of tumor cells*

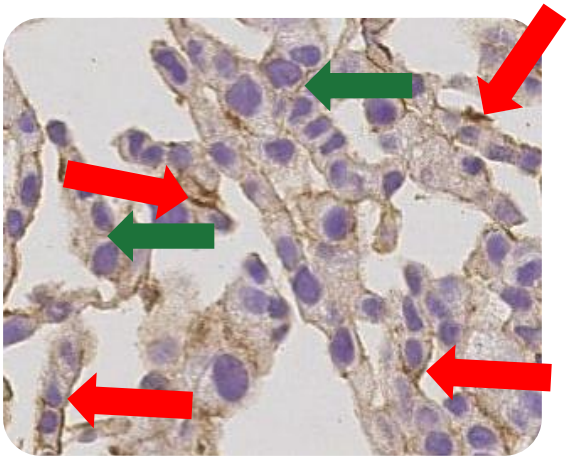
Generally the HistoCyte 2+ cell line is more like (a) from the HER2 guidelines while other cells lines such as the 2+ in the NEQAS cell lines are more akin to (b). However, these are guidelines and the cell staining can vary slightly with each assay. Nuclear staining is aberrant and should be ignored (orange arrow). As should excessive cytoplasmic staining (red arrow) which is more pronounced with some assays. Genuine membrane staining should be scored and can be very subtle with some assays (blue arrow).

1+ Cell line staining

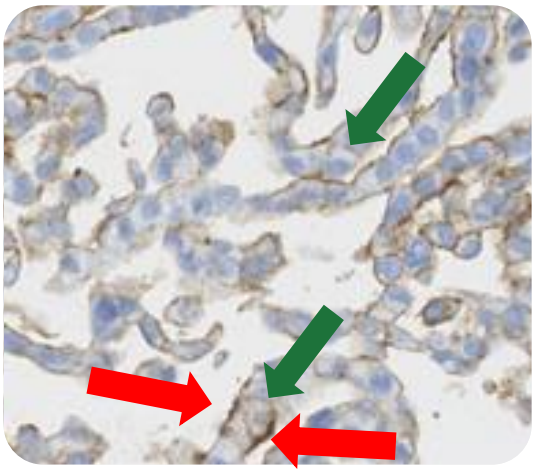
Roche/Ventana



Leica Biosystems

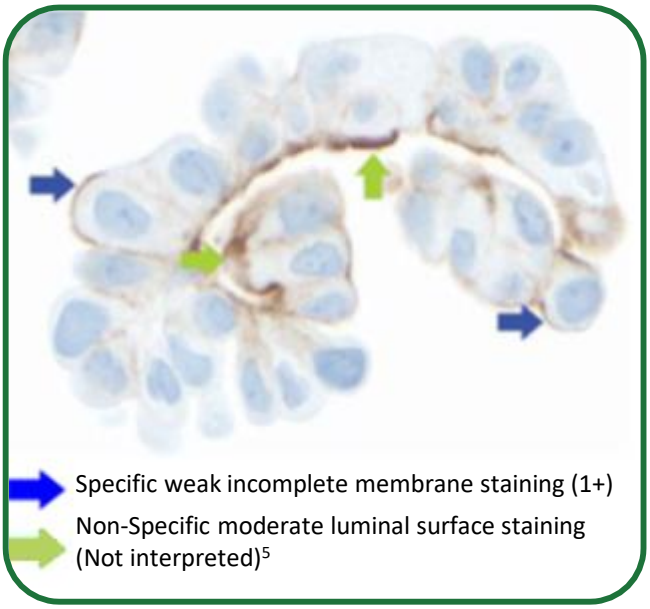


Agilent



HER2 Guidelines 1+
Incomplete membrane staining that is faint/barely perceptible and within >10% of tumor cells

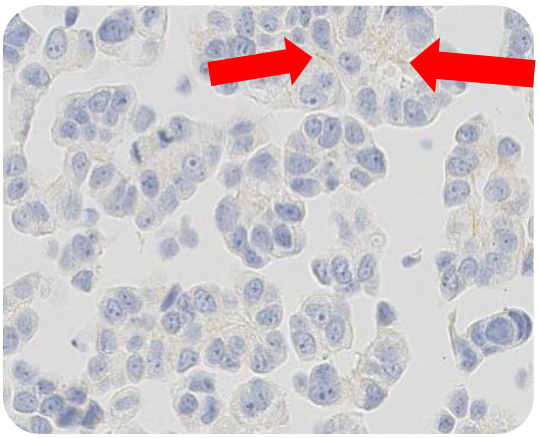
The brush border is more apparent with some assays over others. It is an observation previously noted in other cells such as those used by UKNEQAS.[5]



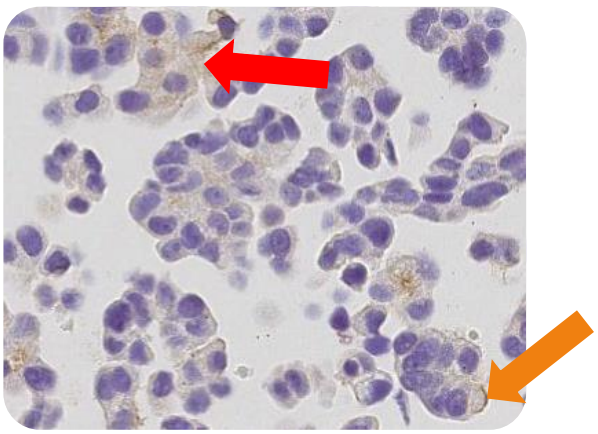
The brush border is not counted. This is the same with the HER2 Analyte Control[DR] (red arrows). Genuine membrane staining is seen between adjoining cells (green arrows).

0 Cell line staining

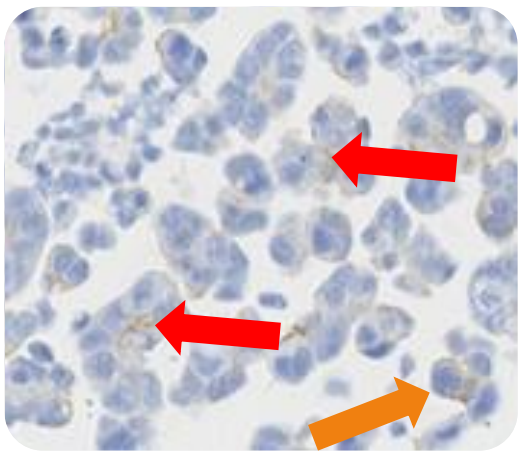
Roche/Ventana



Leica Biosystems



Agilent



HER2 Guidelines 0

No staining is observed*

Or

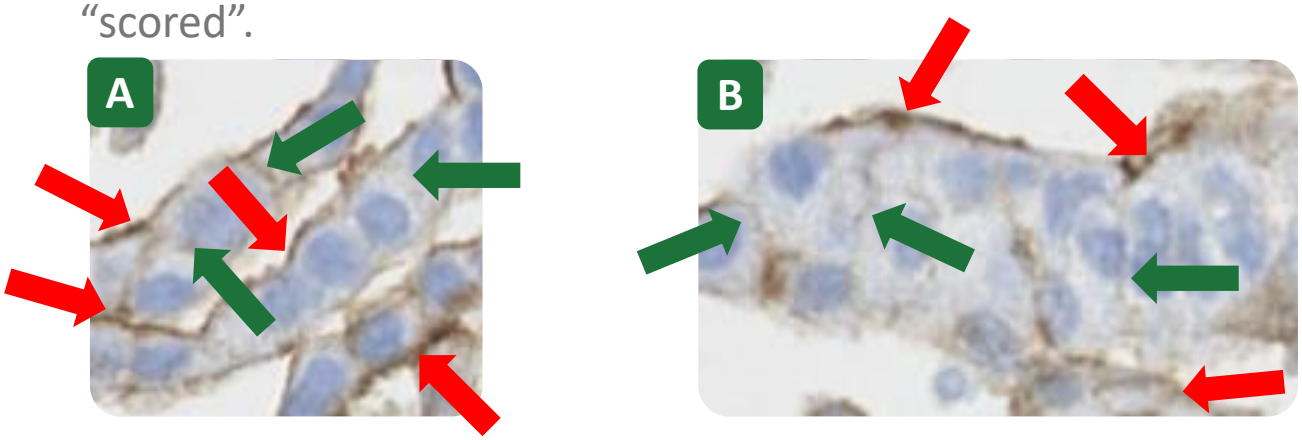
Membrane staining that is incomplete and is faint/barely perceptible and within ≤10% of tumor cells

There should be little or no genuine membrane staining. However, the brush border (BB) of the "clumps" often stain to varying degrees (Red arrows) . Staining on the periphery could be considered (Orange arrows) but still insignificant and likely BB.

Brush Border Explained

Summary

The 1+ brush border is worse with some assays over others and like excessive cytoplasmic staining should not be scored. Only where cells are adjoining should the membranes be "scored".



The red arrows indicate the brush border. The green appears to be genuine membrane staining. This cell "clump" (B) has brush border coming up (red arrow) but within the clump the cell membranes are negative to weakly positive.

So what is it?

It is important to note that these are glandular/luminal cells with a secretory capacity which localises where there is an absence of cell-cell contact. This is typically only seen in the lumen of the duct (see figure 3a) but as the cells lack any myoepithelial layer the brush border effect is made apparent at the exposed extremities of the cells (see figure 3b). These are cultured clones where there are no neighboring cells.

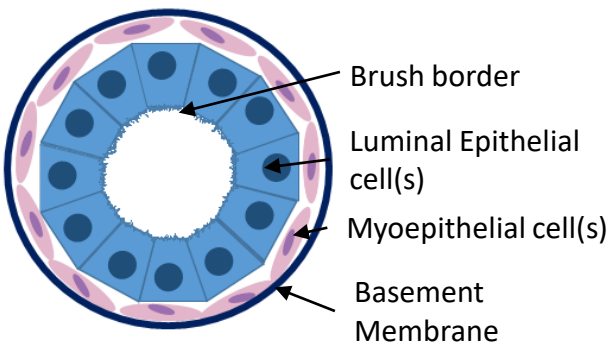
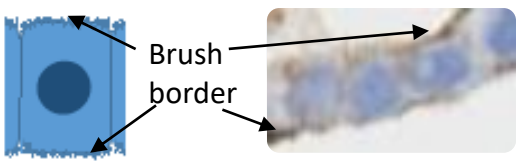


Figure 3a. Breast duct



The secretory capacity of the cell localises at surfaces absent of other cells

Figure 3b. Brush border of breast adenocarcinoma cells.

Also Available From HistoCyte Laboratories Ltd

Product Name	Format	Code
HPV/p16 Analyte Control[DR]: Four cores with a dynamic range of HPV gene copies	Slides (2)	HCL001
	Slides (5)	HCL002
	Block	HCL003
HPV/p16 Analyte Control: Three cores with a standard range of HPV gene copies	Slides (2)	HCL004
	Slides (5)	HCL005
	Block	HCL006
ALK-Lung Analyte Control: Two cores positive and negative for the EML4-ALK translocation	Slides (2)	HCL007
	Slides (5)	HCL008
	Block	HCL009
ALK-Lymphoma Analyte Control: Two cores positive and negative for the NPM-ALK translocation	Slides (2)	HCL010
	Slides (5)	HCL011
	Block	HCL012
Breast Analyte Control: Two cores, one positive for HER2, ER and PR, the other negative	Slides (2)	HCL013
	Slides (5)	HCL014
	Block	HCL015
Breast Analyte Control[DR]: Five cores with a dynamic range of expression of HER2, ER and PR, including a negative control	Slides (2)	HCL016
	Slides (5)	HCL017
	Block	HCL018
PD-L1 Analyte Control[DR]: 4 cores with a dynamic range of PD-L1	Slides (2)	HCL019
	Slides (5)	HCL020
	Block	HCL021
ROS1 Analyte Control: Two cores positive and negative for ROS1 translocation	Slides (2)	HCL022
	Slides (5)	HCL023
	Block	HCL024
Sienna Cancer Diagnostics hTERT assay. 1ml of anti-hTERT mouse mAb. *(Available UK & Ireland Only)*	1ml	HCL025
HER2 Analyte Control[DR]: Four cores, 0, 1+ (both non-amplified), 2+ (equivocal) and 3+ (amplified)	Slides (2)	HCL026
	Slides (5)	HCL027
	Block	HCL028