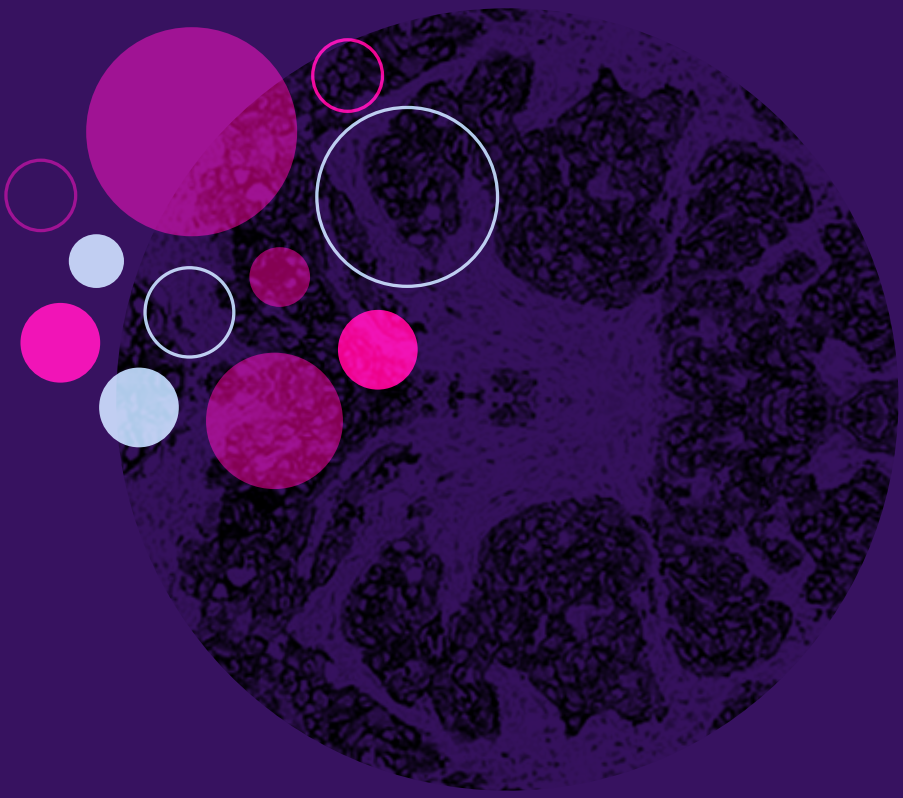
HER2 GLOBAL DIAGNOSTICS

HER2 IHC SCORING GUIDE:
INTERPRETATION OF STAINING IN BREAST CARCINOMAS



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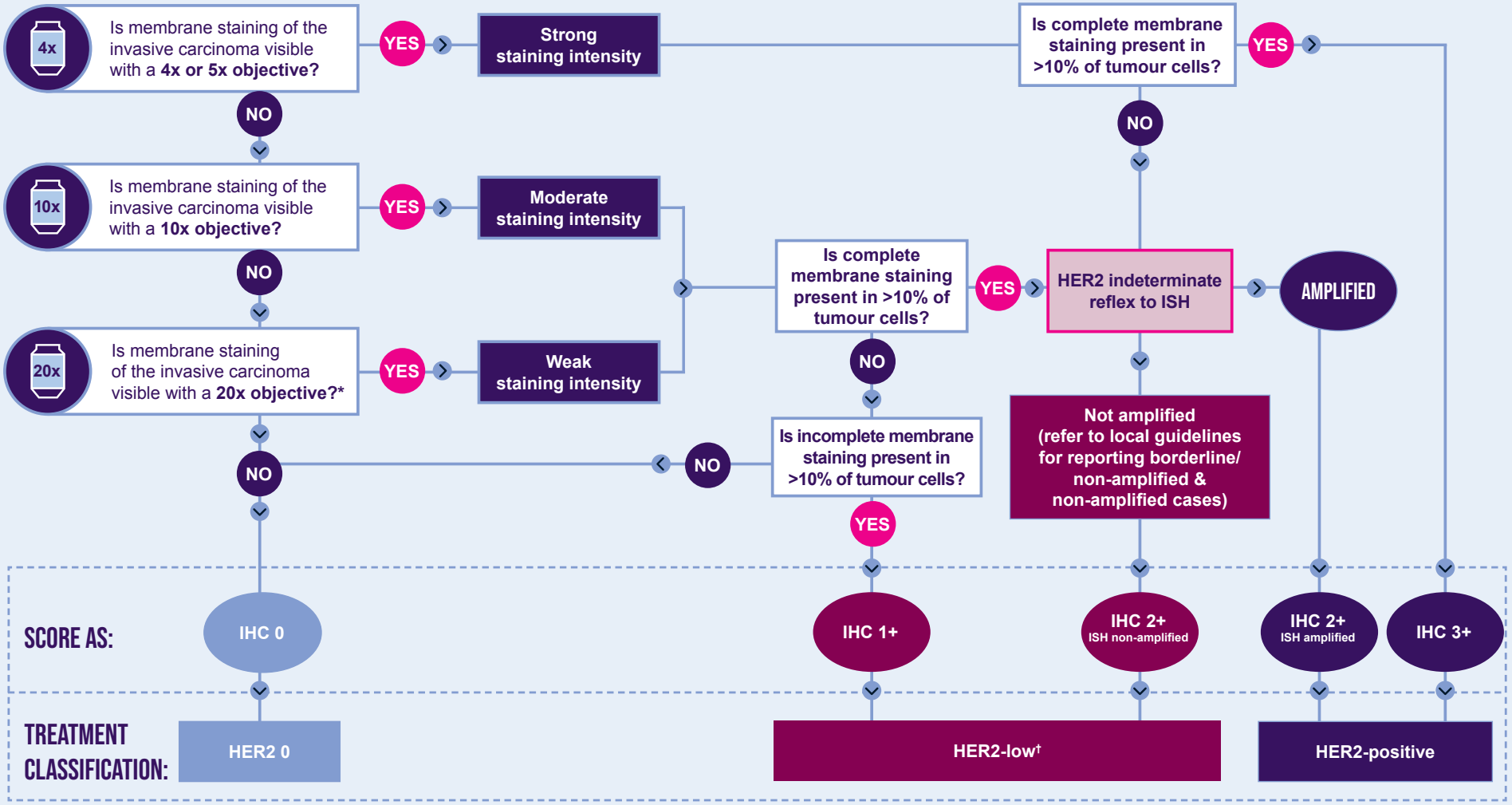
SCORING ALGORITHM

This algorithm was developed in collaboration with Dr Corrado D'Arrigo and the team at Poundbury Cancer Institute, UK.

Determine HER2 status (invasive component) by a validated IHC assay on any invasive breast carcinoma at the time of diagnosis or at relapse. Ensure appropriate staining of on-slide controls before proceeding[1,2]



40x

*In some cases, it may be desirable to use the 40x objective to confirm the level of membrane staining. If staining is only visible at x40, discretion is needed to avoid over-scoring

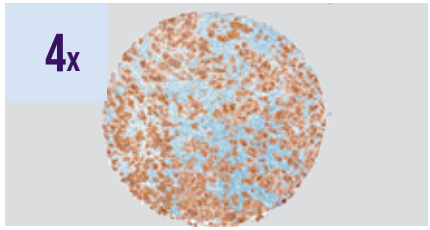
†Assuming >10% complete or incomplete membrane staining of any intensity for IHC 2+/ISH not amplified.

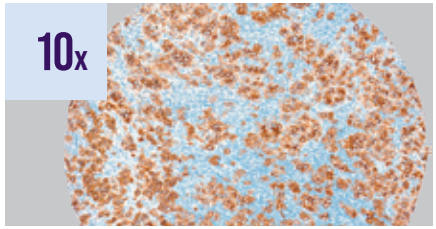
SCORING PARAMETERS

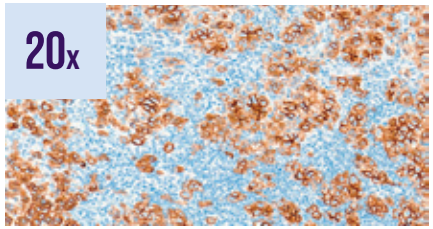
1 STAINING INTENSITY

Use the magnification rule (see below) for accurate measurement of line thickness; it may be necessary to examine some cases with a 40x or higher objective to ensure that there is no membrane staining

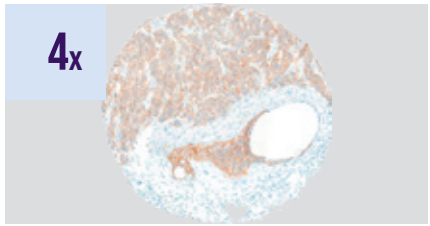
Strong staining: membrane staining visible with 4x or 5x objective

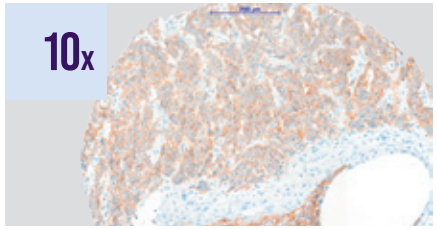


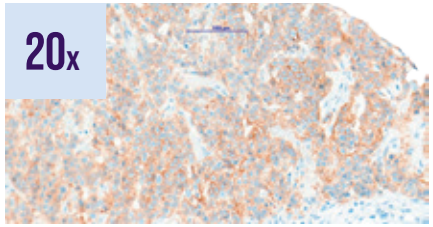




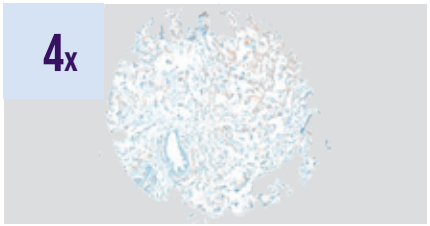
Moderate staining: membrane staining visible with a 10x objective

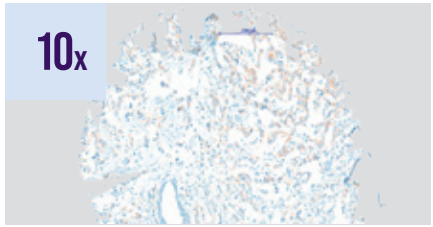


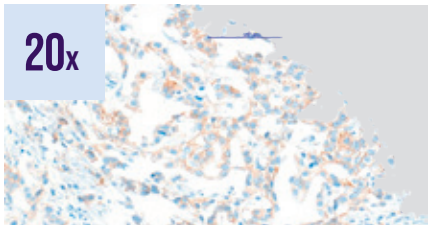




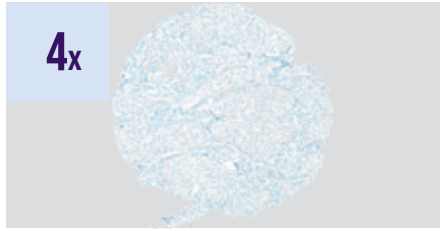
Weak staining: membrane staining visible with a 20x objective

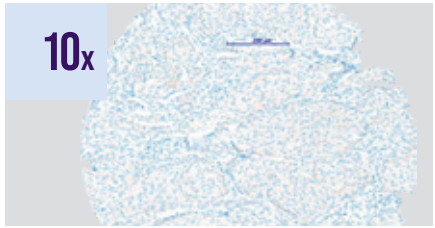


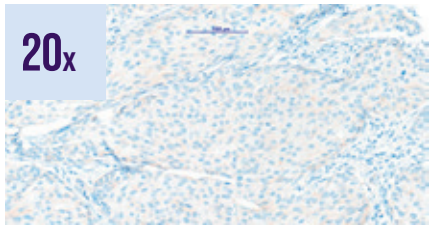




No staining: membrane staining not visible with a 20x objective







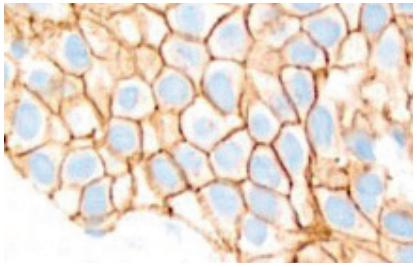
MAGNIFICATION RULE[3]

- Membrane staining recognisable with 4x–5x objectives: IHC 3+
- 10x–20x objectives needed to unequivocally identify membrane staining: IHC 2+
- Any staining that is visible only with a 20x objective may need 40x for confirmation: IHC 1+

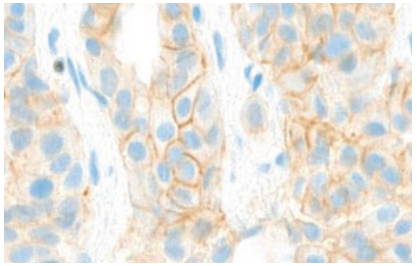
SCORING PARAMETERS

2 STAINING PATTERN

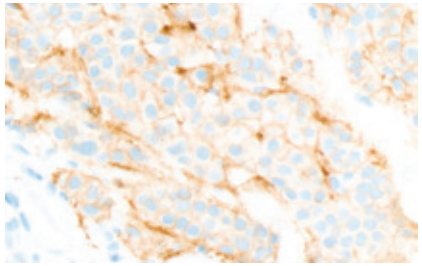
Complete (circumferential)



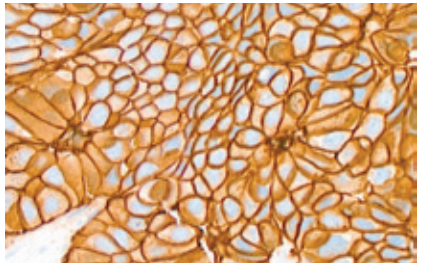
Complete (circumferential)



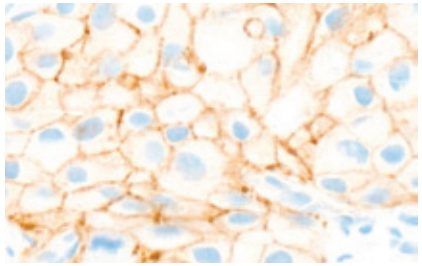
Incomplete (partial)



Incomplete (partial)

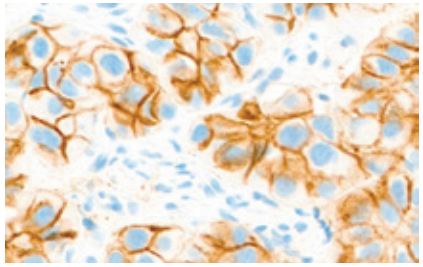


Chickenwire

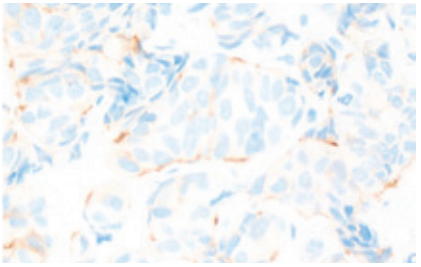


Beads on a string

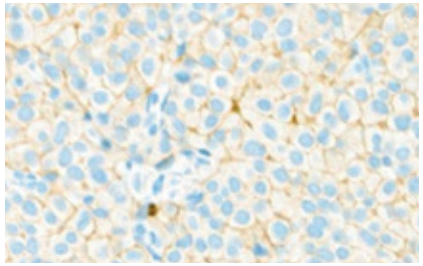
Acceptable staining patterns: linear complete (basolateral), linear incomplete, beads on a string, crow feet.[1-2] **Unacceptable staining patterns:** diffuse cytoplasmic, granular cytoplasmic, basal membrane-like staining, luminal or extracellular only.[4] **Interpret with caution:** basolateral, diffuse granular[4]



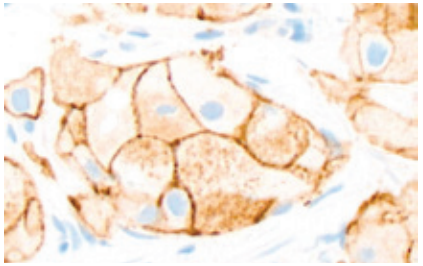
Basolateral



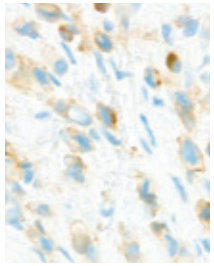
Basal membrane-like staining



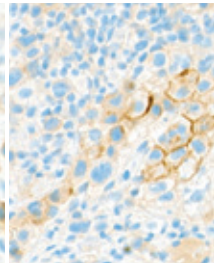
Crow feet (dotted/patchy)



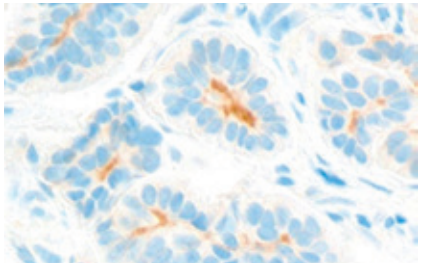
Diffuse granular



Cytoplasmic (focal)



Cytoplasmic (diffuse)



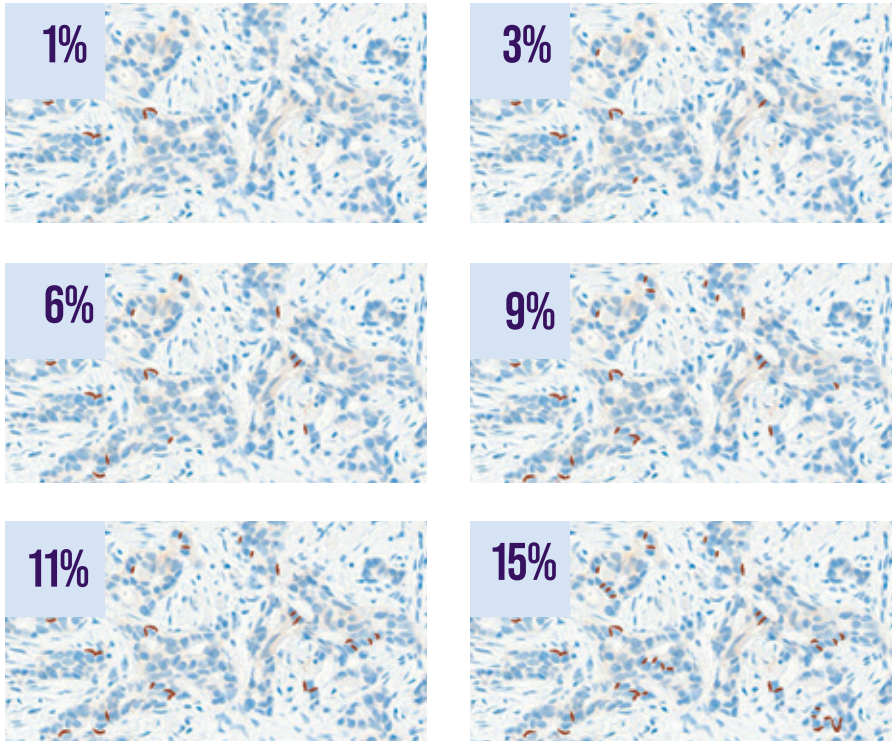
Luminal extracellular staining

SCORING PARAMETERS

3 PERCENTAGE TUMOUR CELL STAINING

Counting method in cases falling at the 10% cut-off: Count positively and negatively stained tumour cells in areas with densely packed medium sized tumour cells with a 20x objective field ~2.500

The following examples serve to distinguish between <10% and >10% tumour cell staining in determining HER2 diagnosis. **Note: these are mock-up images and actual images may differ**

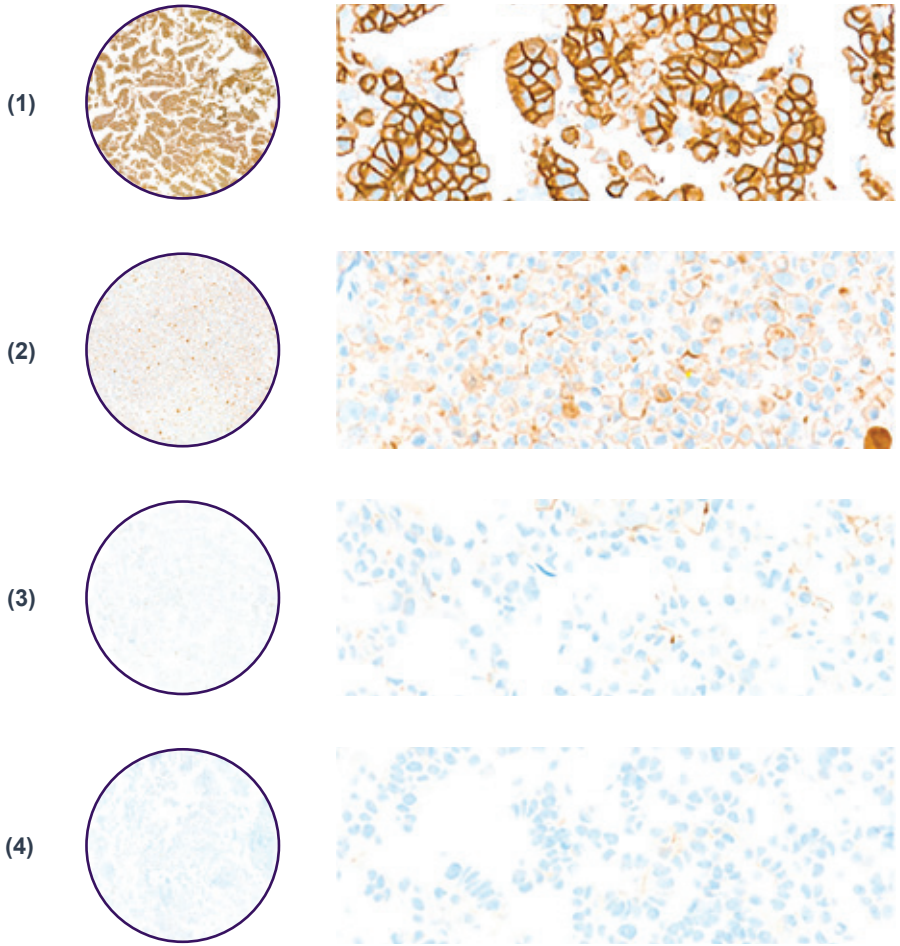


INTERPRETATION EXAMPLES

CONTROLS CHECK

Good analytical performance: There is good staining in the strong core (1) and only weak apical cytoplasmic/no membrane staining (4). There is little staining in the moderate core (2) and staining in the weak core (3) can only be seen at 20x

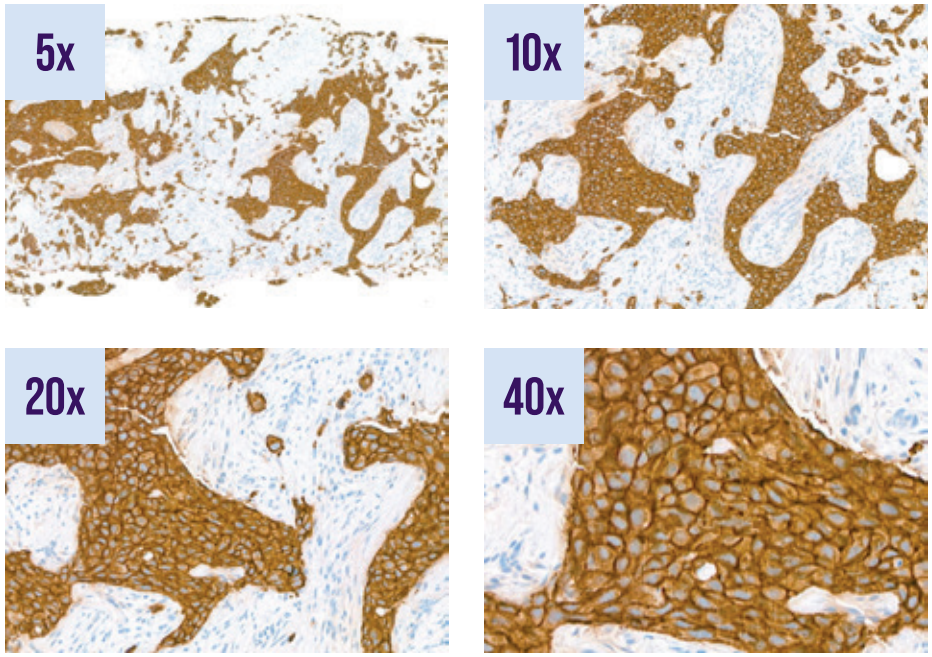
Proceed with assessment



INTERPRETATION EXAMPLES

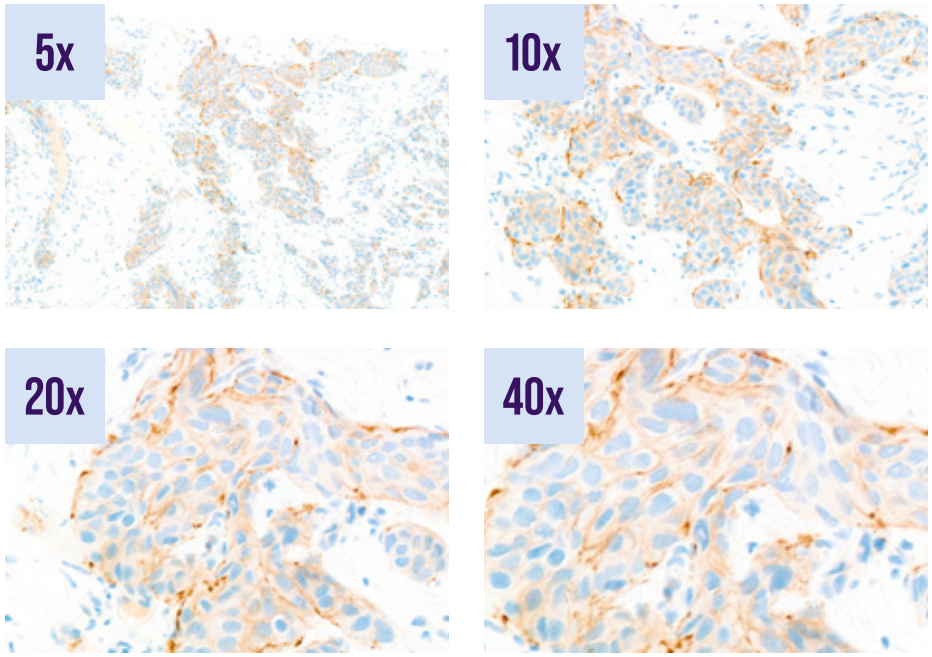
CASE STUDIES

Real-world examples and associated IHC scores. Case images captured with 5x, 10x, 20x and 40x objectives



CASE 1

- Complete membrane staining visible in all cells at 4x or 5x (strong staining intensity)
- **IHC score:** 3+
- **HER2 classification:** HER2-positive



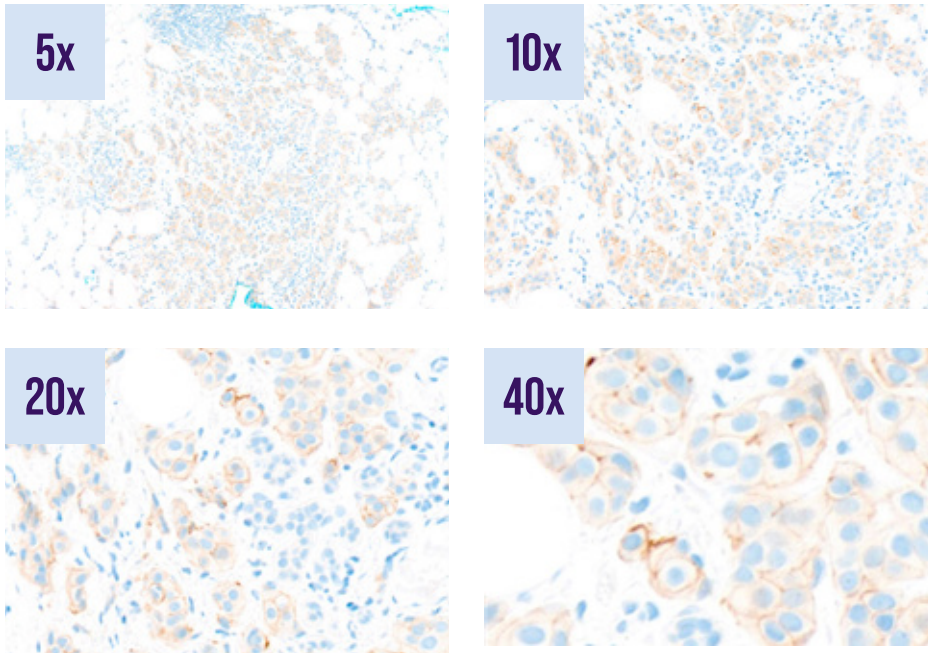
CASE 2

- Discontinuous membrane staining visible at 5x (strong staining intensity); this is sufficient for an IHC score of 2+. At 10x and 20x more membrane staining becomes visible (moderate and weak staining intensity, respectively) and some of this membrane staining appears circumferential. This is best observed at 40x
- **IHC score:** 2+
- **Preliminary HER2 classification:** HER2 indeterminate, reflex to ISH

INTERPRETATION EXAMPLES

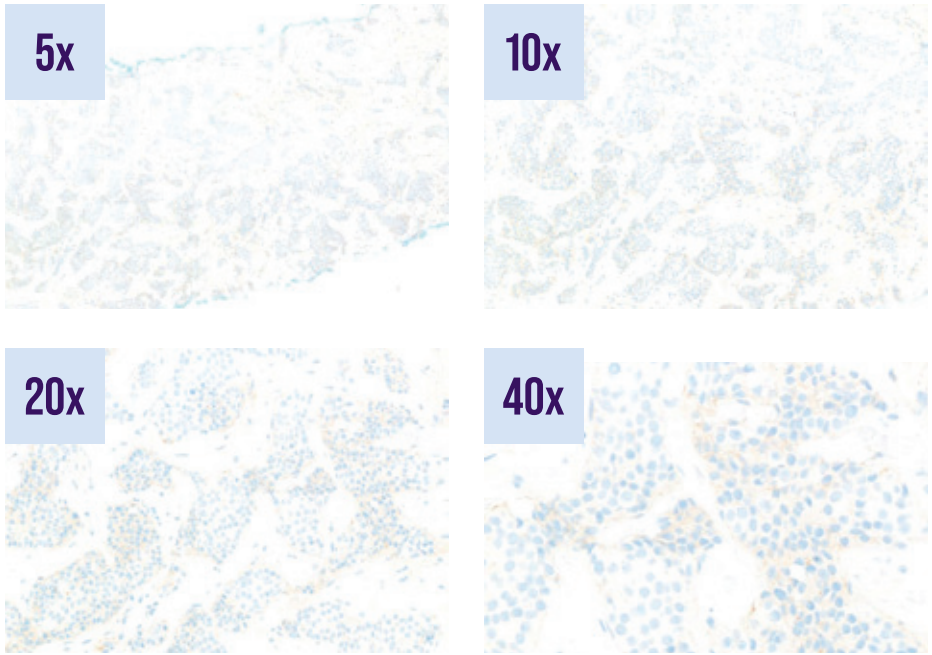
CASE STUDIES

Real-world examples and associated IHC diagnoses



CASE 3:

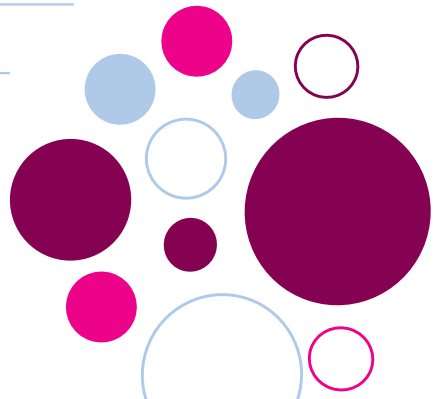
- At 5x there is vague staining that cannot be localised to the membrane. At 10x there are a few cells with discontinuous membrane staining (moderate staining intensity). At 20x more cells show membrane staining; however, this remains discontinuous. Observation at 40x confirms that membrane staining is not circumferential
- **IHC score:** 1+
- **HER2 classification:** HER2-low

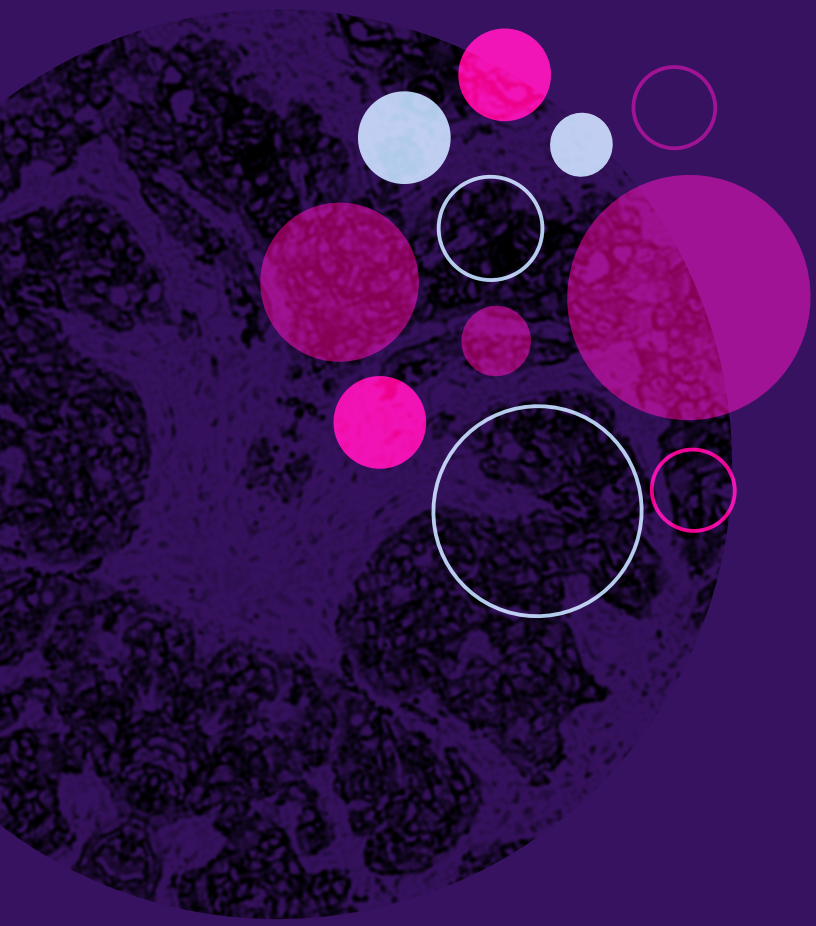


CASE 4:

- Even with 40x, only some weak cytoplasmic membrane staining visible
- **IHC score:** 0
- **HER2 classification:** HER2-null

NOTES





This scoring algorithm has been approved by Dr. Annette Lebeau, Dr. Corrado D'Arrigo and Dr. Joseph Rüschoff

Developed in collaboration with, and images provided by, Dr Corrado D'Arrigo and the team at Poundbury Cancer Institute, UK

Abbreviations:
HER2=human epidermal growth factor receptor 2; IHC=immunohistochemistry; ISH=*in situ* hybridisation; TC=tumour cell.

References:

1. Wolff AM, et al. *J Clin Oncol.* 2023. doi: 10.1200/JCO.22.02864.
2. Wolff AM, et al. *J of Clin Oncol.* 2018;36(20):2105–2112.
3. Scheel AH, et al. *Diagn Pathol.* 2018;13(1):19.
4. Grassini D, et al. *Pathobiology.* 2022;89(5):278–296.

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