Her-2 neu:

The sections to be stained for HER comprised:

No.	**Tissue**	**Her score (0,1+,2+,3+)**	**FISH/CEP ratio**	**FISH result**
1	Breast ca	3	13.75000	Positive
2	Breast ca	2	1.488372	Negative
3	Breast ca	2	1.121212	Negative
4	Breast ca	1	1.571429	Negative
5	Breast ca	2	7.000000	Positive
6	Breast ca	1	1.789474	Negative

All tissues sent were fixed in 10% neutral buffered formalin for 24 to 48 hours.

The selected cases were also tested for FISH amplification. Gene amplification status was taken into account for fair assessment of the marker and address any bias in staining.

Scoring of HER-2 immunohistochemistry:

The most widely used scoring method for HER-2 IHC in breast cancer is semiquantitative and based of four classes (0/1+/2+/3+). The scoring can be verbalised as follows:

Score 0 No staining is observed or cell membrane staining is observed in less than 10% of the tumour cells. (“negative”).

Score 1+ A faint perceptible membrane staining can be detected in more than 10% of the tumour cells. The cells are only stained in part of their membrane. (“negative”).

Score 2+ A weak to moderate complete membrane staining is observed in more than 10% of the tumour cells. (“weakly positive”).

Score 3+ A strong complete membrane staining is observed in more than 30% of the tumour cells.

Criteria for assessing a HER-2 staining as **optimal** were:

- Staining corresponding to score 3+ in carcinoma no.1
- Staining corresponding to score 2+ or 3+ in carcinomas no. 5.
- Staining corresponding to score 1+ or 2+ in rest of the cases
- No or only a weak cytoplasmic reaction that did not interfere with the interpretation

A staining was assessed as **good**, if

- the HER-2 gene amplified tumor nos. 1 and 5 showed a 2+ reaction and the other breast carcinomas showed reaction pattern as described above or
- the HER-2 gene nonamplified tumors showed a 2+ or less reaction.

A staining was assessed as **borderline** if noise level was high, e.g., because of cytoplasmic reaction, excessive counterstaining or excessive retrieval hampering the interpretation.

A staining was assessed as **poor** in case of a false negative staining of carcinoma case no. 1 and 5

Results:

Total 15 laboratories participated in this run:

Optimum	3
Good	1
Borderline	1
Poor	9
Rejected	1

Antibody details and performance analysis:

RTU	Conc	Vendor	N	Clone	Optimal	Good	Borderline	Poor
9		Biogenex	1	CB11		1		
		Dako	1	Polyclonal	1			
		Biogenex	2	EP1045Y				1
		Cellmarque	1	CB11			1	
		Ventana	1	4B5				1
		Gennova	1	SP3				1
		Path in situ	1	EP3				1
		Leica	1	CB11				1
		Dako A0485	3	C-erb B2	2			1
		Labvision	2	SP3				2

		Biocare	1	EP1045Y				1

Comments:

1. The most prominent feature of insufficient HER staining was weak or negative staining reaction.
2. This was notable in several instances. The important situations were:
    - Case 1/3+/Amplified; 5 laboratories identified them as 3+ and 4 laboratories identified them as score 2+. This case was positive in our control study as well as having greater FISH ratio too. This may unnecessarily subject the cases to FISH testing and also diminish confidence of the oncologist in the testing.
    - We removed case 3 from the statistical analysis. However, 3 laboratories did get it 2+ score. This should not be disregarded, particularly when FISH analysis had been positive in that case. 5 laboratories reported this to be 0 and 1 laboratory as 1+. This case would be deprived of anti-Her therapy as the oncologist would not go for FISH testing in this situation.
    - Case no. 4 was reported as 2+ by 3 laboratories, 1+ by 2 and 0 by 4 laboratories. This was tested negative by FISH. The FISH ratio in that case was 1.7. The laboratories testing this as 2+ were doing so because of good sensitivity of their testing and probably rightly scoring keeping with the FISH results in that case.
3. With regards to comment no. 2 it should be noted that the 'underlined 3 laboratories' were same throughout. These 3 laboratories were concurring with one another in all the cases and they were having most consistent results with regards to FISH testing. This indicates that they employed better sensitive protocol.
4. These 3 laboratories used clone c-erb B2. Two of them used RTU and one used concentrated. The concentrated antibody was diluted at 1:700.
5. HIER was used by all laboratories.
6. Pressure cooker and MWO both were used equally in the group. Two participants used decloaking chambers. Best results were obtained with MWO.
7. Citrate at pH 6.0 was used by 2 laboratories. They were having more insufficient results. Those 3 laboratories used Tris-EDTA at pH 9.0 and used MWO for HIER.
8. The range of dilution had been from 1:50 to 1:1000. The range for Dako antibodies had been 1:600 to 1:1000.
9. Cytoplasmic granular staining affected testing in case of 1 laboratory.

Recommended protocol for HER testing

Obtained in Breast Module, run 1

Primary antibody

Clone	c-erb B2
Producer	Dako
Product no. (Lot no.)	00045398
Dilution	1:700
Diluent buffer and additive(s)	Dako antibody diluent
Incubation time / temperature	30 min./RT

Epitope retrieval, HIER

Device	Manual
Buffer, pH	Tris-EDTA based buffer, pH 9.0
Warm-up / heating max / resting time	Peak temperature of 95 degree for 20 min in MWO

Visualization system

Method	Polymer conjugate
Producer, product no.	Dako Envision plus
Incubation time / temperature	30 min./RT

Chromogen

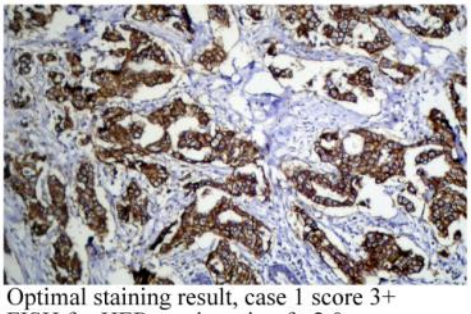
Type	DAB
Enhancement, type	Copper sulphate

Immunostainer

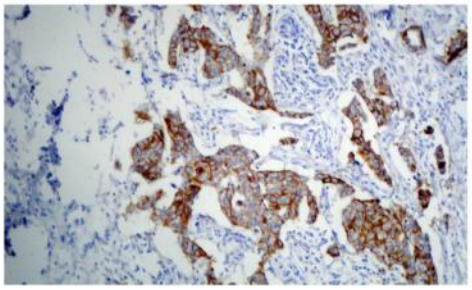
Type	Manual

Abbreviations HIER: Heat induced epitope retrieval, RT: Room temperature

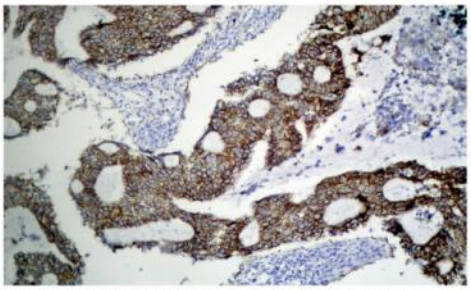
Images in following pages:



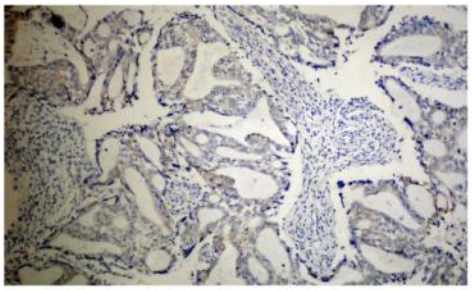
Optimal staining result, case 1 score 3+ FISH for HER pos in ratio of >2.0. >10% tumor cells with strong and com lete membrane staining



Insufficient staining of case 1. >10% cells show complete membrane staining of moderate intensity.

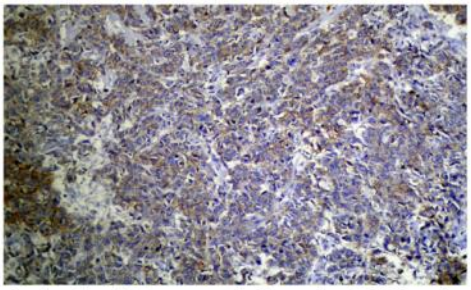


Optimal staining case 3, FISH ratio of 1.7. Moderate staining of >10% of the tumor cells with complete membrane stain.

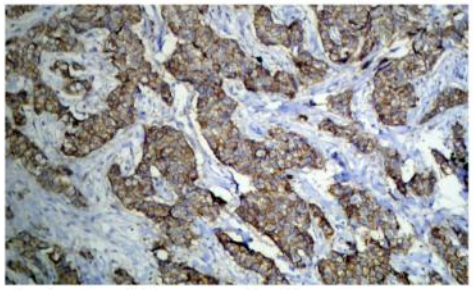


Insufficient staining of case 3. Note absence of staining of the tumor cells

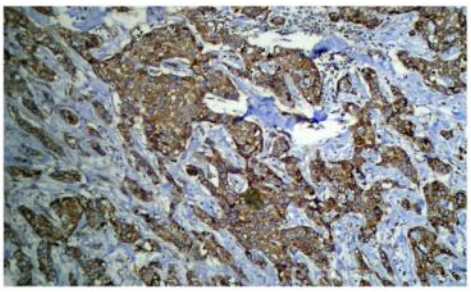
P.T.O.

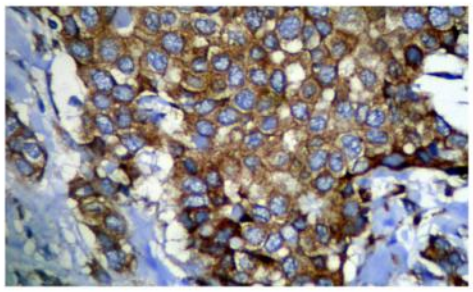


Optimal staining, case 5. Score 1+. FISH ratio <1.0. Note faint staining of part of membrane in >10% of tumor cells.



Optimal staining, score 2+. Case 6. FISH ratio of <2.0





Cytoplasmic granular staining impairing fair interpretation of the case.