Estrogen receptor-alpha

The sections to be stained for ER comprised:

No.	Tissue	ER positivity	ER intensity
1	Breast ca	2-5%	Weak to mod
2	Uterine cervix	80-90%	Mod to strong
3	Breast ca	5-10%	Weak to mod
4	Breast ca	>90%	Mod to strong
5	Breast ca	0%	
6	Breast ca	0%	

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

Criteria for assessing staining as **optimal** were:

- Moderate to strong, distinct nuclear staining reaction of virtually all columnar epithelial cells, basal squamous epithelial cells and most stromal cells (except endothelial and lymphoid cells) in the uterine cervix.
- At least moderate and distinct nuclear staining reaction in the appropriate proportion of the neoplastic cells in the breast carcinomas no. 4.
- At least weak staining in breast carcinoma in no.3 and no.1
- No nuclear staining reaction of neoplastic cells in the breast carcinoma no. 5 and 6.
- No more than a weak cytoplasmic staining reaction in cells with strong nuclear staining reaction.

The staining reactions were classified as **good** if ≥ 10 % of the neoplastic cells in the breast carcinomas no. 4 showed an at least weak nuclear staining reaction.

The staining reactions were classified as **borderline** if ≥ 1 % and < 10 % of the neoplastic cells showed a nuclear staining reaction in breast carcinoma no. 4

The staining reactions were classified as **poor** if false negative result in case 4 or false positive staining reaction was seen in one of the breast carcinomas.

Results:

Total 9 laboratories participated in the assessment. Six laboratories achieved optimum results while 3 achieved good results.

Antibody details and performance analysis:

RTU	Conc	Vendor	N	Clone	Optimal	Good	Borderline	Poor
6		Dako	3	EP1	2			1
		Ventana	1	SP1	1			
		Cellmarque	1	SP1	1			
		Biogenex	1	1D5			1	
	3	Biocare	1	SP1	1			
		Thermo	1	SP1		1		
		Dako	1	EP1	1			

Comments:

Criteria for assessment were kept lenient in this run keeping in view the therapeutic use of the marker in question. The assessment was centered on the case no. 4 that showed good degree of positivity overall.

However, the weak staining cases were particularly kept for assessment of the overall sensitivity of the protocols employed. For case no. 1 where staining was expected in fewer cells in weak to moderate degree, only 4 laboratories had optimum results and 3 had no staining at all. Slightly higher proportion of positivity was kept in case 3 for which all, except 3 laboratories, had optimum results.

It was observed that intensity of staining was rather weak in majority of participants in both, cervix and case 4.

We had clones EP1, SP1 and 1D5 in this assessment. **SP1 and EP1 were the most successful clones**. The best results were obtained with clone SP1 from Ventana, Benchmark automated system with RTU.

All laboratories used HIER, using MWO or pressure cooker. One laboratory used decloaking chamber while one laboratory used Benchmark system. No significant difference in result was found with that.

6 out of 9 laboratories used tris-EDTA buffer at pH 9.0. Two of them used company formulas, both at alkaline pH. One laboratory used citrate buffer at pH 6.0. This particular participant had borderline performance. It is seen in several other studies also that **HIER in alkaline pH yields best results**.

The three laboratories using concentrated antibodies had dilutions 1:50 (Biocare, SP1), 1:100 (Dako, EP1) and 1:300 (Thermo, SP1). However, the one with Thermo, SP1 and having highest dilution had sub-optimal performance. **Too much dilution has interfered in staining reaction that was weak**, overall, in that case.

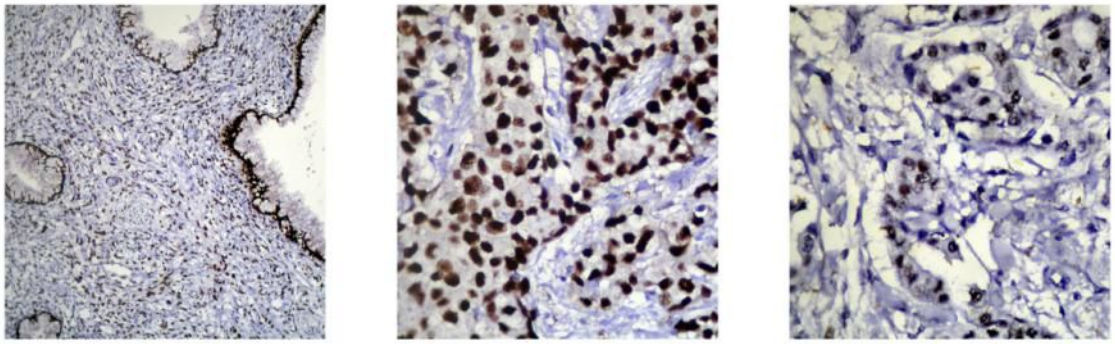
Uterine cervix is an appropriate positive tissue control for ER. Virtually all stromal, columnar epithelial and squamous epithelial cells must show a moderate to strong and distinct nuclear staining reaction.

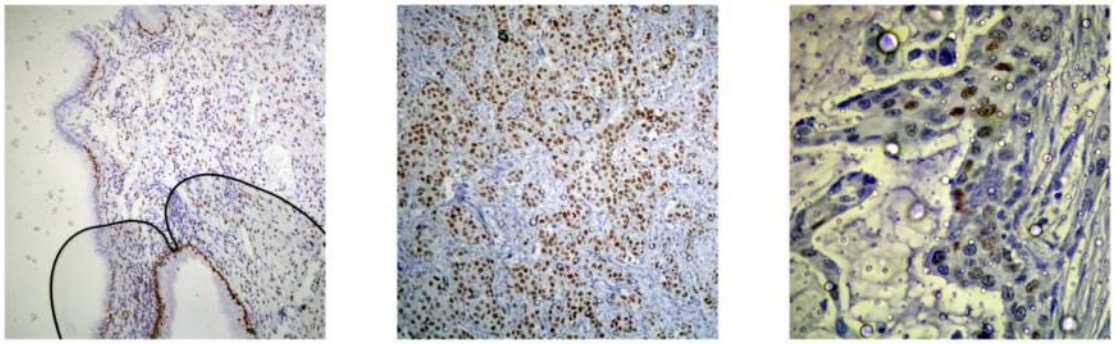
Lymphocytes and endothelial cells must be negative. In this run, staining reaction of cervix had good concordance rate with overall staining. We therefore recommend cervix as an appropriate control.

In order to validate the specificity of the IHC protocol, **ER negative breast carcinoma must be included** in which only remnants of normal epithelial and stromal cells must be ER positive serving as internal positive tissue control. Positive staining reaction of the stromal cells breast tissue indicates that a high sensitive protocol is being applied, whereas the sensitivity cannot be evaluated in the normal epithelial cells as they express high levels of ER.

Images in next pages:

Optimal protocol setting. The cervix shows strong staining of the columnar epithelial cells as well as stromal cells. The same lab shows strong staining of case 4 and moderate intensity staining in case 3.





Insufficient protocol setting. This lab shows weaker staining reaction in cervical epithelial and stromal cells. The same lab showed weaker staining in case 4 and case 3. Compare with the above case.

The laboratories that showed weaker staining in cervix and case 4, failed to detect the weak positive cases nos. 1 and 3. This was not strictly accounted for in the assessment; however this may affect the overall sensitivity of the testing.