Breast module. Cycle 1, Run 2

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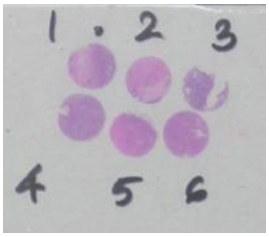
May 2015



The sections to be stained for ER comprised :

No.	Tissue	ER positivity	ER intensity
1	Breast ca	0%, internal control	Negative. Strong internal control
2	Uterine cervix	80-90%	Mod to strong
3	Breast ca	0%	Negative
4	Breast ca	30-40%	Weak to moderate
5	Breast ca	80-90%	Moderate to strong
6	Breast ca	80-90%	Moderate

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 weak to moderate and distinct nuclear staining reaction in the appropriate proportion of the neoplastic cells in the breast carcinomas no. 4.
- Positive internal control in case 1
- No nuclear staining reaction in case 1 and 3 in tumor cells
- Appropriate degree of staining in cases 5 and 6

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

The staining reactions were classified as **borderline** if ≥ 1 % and < 10 % of the neoplastic cells in case 4 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.

Participation:

Fifteen, 15 laboratories participated

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Results:

	No. of labs (total)
Optimum	8
Good	3
Borderline	0
Poor	4

Antibody details and performance analysis

RTU	Conc	Vendor	N	Clone	Optimal	Good	Borderline	Poor
9		Dako	4	EP1	4			
		Ventana	1	SP1				1
		Cellmarque	1	SP1	1			
		Biogenex	2	1D5				2
		Leica	1	6F11				1
	6	Labvision	2	SP1	1	1		
		Dako,M7047	1	1D5		1		
		Dako,M3643	2	EP1	2			
		Biocare	1	SP1		1		

Comments:

1. **Which clone has the best outcome?**

   Clone EP1 (Dako) yielded best performance statistics in RTU as well as Concentrated formats. All six laboratories using this had optimum performance (100%). All the laboratories used it with HIER at alkaline pH. Five laboratories had manual method while one laboratory used it with Ventana Benchmark XT platform.

   Clone SP1 was used by total five laboratories (2-optimum, 2-good and 1-poor). This antibody was from different vendors. The laboratory with poor outcome used Ventana clone with Ventana Benchmark GX platform.

   Results of clone 1D5 and 6F11 were not so promising in this run.

2. **Which vendor has the best performance?**

   All EP1 clones were supplied by Dako. Clone SP1 had different vendors.

3. **What was better, RTU or Concentrated?**

   The results were better with concentrated antibodies as seen in the table above. All poor results were seen with RTU antibodies.

4. **What is the best dilution ratio of the primary antibody?**

   The range of dilution was varied in optimum results. For clone EP1 it was 1:50 to 1:200 while in case of clone SP1 it was 1:300

5. **Has dewaxing temperature to do something with staining reaction?**

   No significant difference was found.

6. **Is the pH of retrieval buffer important?**

   Only 1 laboratory used acidic pH yielding Good result.

7. **What is the best epitope retrieval method?**

   HIER was used by all laboratories.

8. **Which technique was better, manual or automated?**

   Three laboratories used automated system (Leica, Ventan GX and Ventana XT). Only one laboratory had optimum results while the other two had poor. The one having optimum results used it with Dako IS084, clone EP1 RTU with company provided retrieval buffer.

9. **In the poor performance cases, what is more responsible, antibody clone or other factors?**

   Antibody clone was the major contributor. It is evident that six out of 8 laboratories that had optimum results used clone EP1 and all laboratories using clone EP1 had optimum results.

10. **Which antibody clones had poor performance?**

    Clones 1D5 and 6F11 had poor performance.

11. **What is the best control material for this marker?**

    Cervix appears to be the best and easily available control material for this marker.

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Recommended protocol for ER for Concentrated antibody

Obtained in Breast Module, Run 2

Primary antibody

Clone	EP1
Producer	Dako
Product no. (Lot no.)	M3643
Dilution	1:200
Diluent buffer and additive(s)	Dako antibody dilutent, S0809
Incubation time / temperature	30 min./RT

Epitope retrieval, HIER

Device	Manual
Buffer, pH	Tris-EDTA buffer at pH 9.0
Peak temperature and time	95 C. 20 minutes in MWO

Visualization system

Method	Polymer conjugate
Producer, product no.	Dako-Real Envision K5007
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

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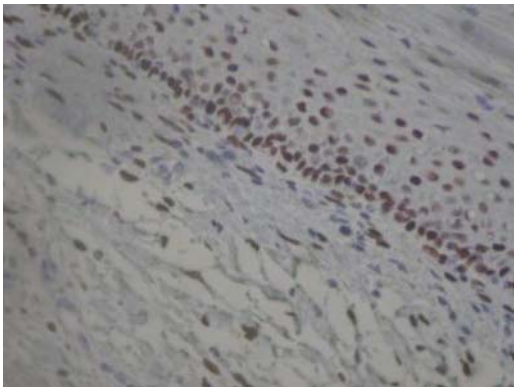

Fig.1: Optimum staining of basal layers of squamous epithelium in cervix. Clone EP1, RTU, Dako IS084 at pH 9.0

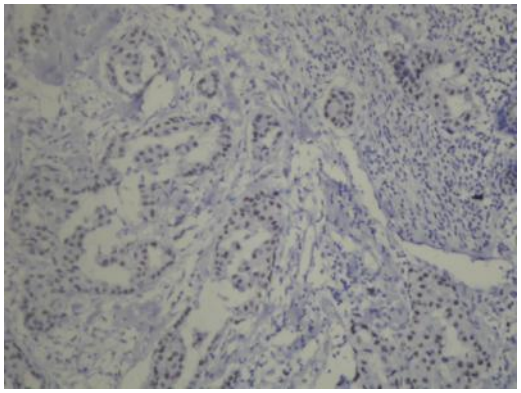

Fig.2: Optimum staining of Case no.4 This lab has optimum protocol setting producing optimum staining of cervix also, like Fig.1

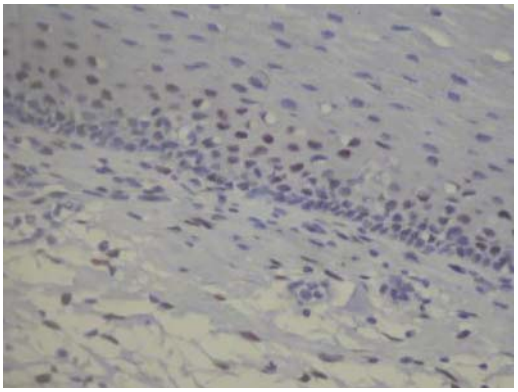

Fig.3: Weak staining of basal layer in cervix. Such intensity of staining would miss low expresser cases like case no.4. Cf. Fig.1

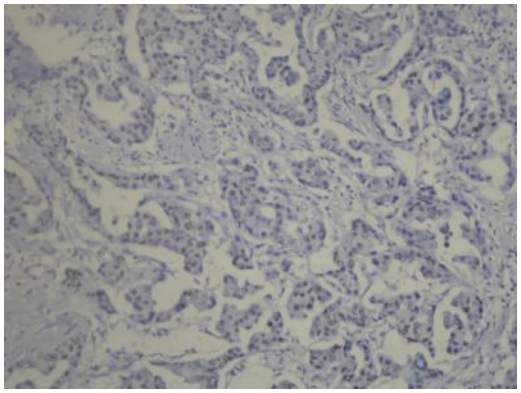

Fig.4: Same lab as Fig.3 and case no.4 Note the weak staining of less number of cells. Compare with Fig.2

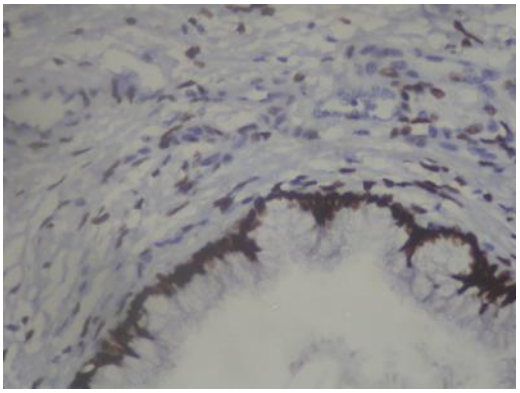

Fig.5: Optimum staining of endocervical mucosa using clone EP1.

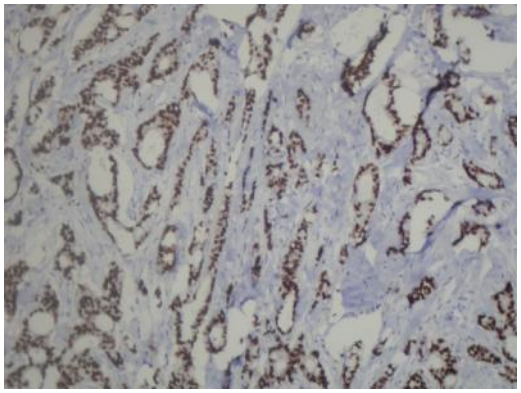

Fig.6: Same laboratory as Fig.5. Case No.5 shows strong staining reaction of tumor cells.