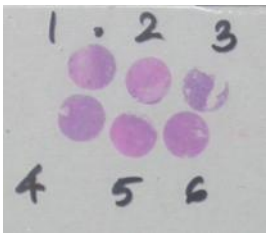

The sections to be stained for PR comprised :

No.	Tissue	PR positivity	PR 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	30-50%	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 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

Results:

	No. of labs (total)
Optimum	10
Good	2
Borderline	2
Poor	1

Antibody details and performance analysis

RTU	Conc	Vendor	N	Clone	Optimal	Good	Borderline	Poor
9		Dako,IS068	4	Pgr636	3			1
		Cellmarque	1	Y85	1			
		Ventana	1	1E2	1			
		Biogenex	2	PR88			2	
		Leica	1	16		1		
	6	Labvision	2	SP2	2			
		Dako,M3569	1	Pgr636	1			
		Biocare	2	SP2	1	1		
		Neomarkers	1	SP2	1			

Comments:

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

   This assessment had overall optimum outcome. Pgr636,SP2,1E2 and Y85 are all good clones.

   Typically the HIER was done in buffer at alkaline pH. Only one laboratory used acidic pH of retrieval buffer and had good outcome..

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

   Performance characteristics were not specific to a particular vendor.

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

   Borderline and poor outcome were seen only with concentrated antibodies.

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

   The range of dilution with optimum results was 1:100 to 1:500. Highest dilution with optimum performance was achieved with Dako antibody.

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 Ventana, clone 1E2 RTU with company provided retrieval buffer.

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

   3 laboratories had either borderline or poor performance. Two of them used clone PR88 from Biogenex. No other laboratory had used this clone.

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

    Clone PR88 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.

Recommended protocol for ER for Concentrated antibody

Obtained in Breast Module, Run 2

Primary antibody

Clone	SP2
Producer	Labvision
Product no. (Lot no.)	RM9102
Dilution	1:400
Diluent buffer and additive(s)	UltrAb Diluent Plus, Lab vision, TA-125-UDX
Incubation time / temperature	60 min./RT

Epitope retrieval, HIER

Device	Manual
Buffer, pH	Tris-EDTA buffer at pH 9.0
Peak temperature and time	100 C. 9 minutes in pressure cooker

Visualization system

Method	Polymer conjugate
Producer, product no.	Dako-Real Envision K5007
Incubation time / temperature	30 min./RT

Chromogen

Type	DAB
Enhancement, type	Nil

Immunostainer

Type	Manual

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