Progesterone receptor

The sections to be stained for PR comprised:

No.	Tissue	PR positivity	ER intensity
1	Breast ca	5%	Weak to mod
2	Uterine cervix	80-90%	Mod to strong
3	Breast ca	<5%	Weak
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 a false negative or false positive staining reaction was seen in one of the breast carcinomas.

Results:

	No. of labs (total 9)
Optimal	8
Good	0
Borderline	1
Poor	0

Antibody details and performance analysis:

RTU	Conc	Vendor	N	Clone	Optimal	Good	Borderline	Poor
5		Dako	2	PgR636	2			
		Ventana	1	1E2	1			
		Cellmarque	1	Y85	1			
		Biogenex	1	PR88			1	
	4	Biocare	1	SP2	1			
		Thermo	1	SP2	1			
		Dako	1	PgR636	1			
		Novocastra	1	SP2	1			

Comments:

Overall, the participants performed well for this marker.

We had clones PgR636, SP2, Y85, 1E2 and PR88 were the clones employed. **All of them were optimum in performance except Biogenex RTU clone PR88**. However, it should be noted that this laboratory has used citrate buffer at pH 6.0 that also could have contributed in the borderline score.

The best results were obtained with clone 1E2 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**.

Four laboratories used concentrated antibodies. The range of dilution is 1:100 to 1:500. All four had optimal results. The **highest dilution of 1:500 was achieved with Novocastra clone SP2** using HIER in MWO with 95 degree peak temperature for 20 min, using Envision plus, Dako detection system.

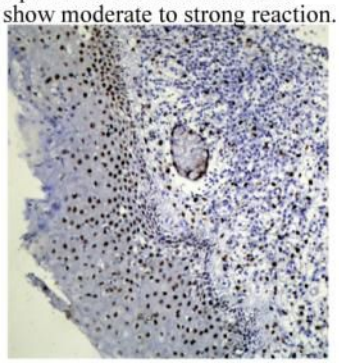
Cytoplasmic staining was observed with clone PgR636 from Dako (both, RTU and concentrated). This did not interfere in interpretation. However, the laboratory must be aware of this fact.

Uterine cervix is an acceptable positive tissue control for PR. 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

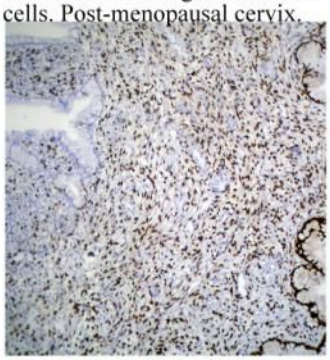
our run, it was found that certain areas of columnar epithelium and squamous epithelium (received by few labs only) showed weaker or no staining. It was found that we used the cervix of a postmenopausal woman. This could have resulted in such occurrence. So, **ideally cervix of a woman of reproductive age groups should be used as control. PR negative breast carcinoma could be an appropriate negative control.**

Images of participants' cases:

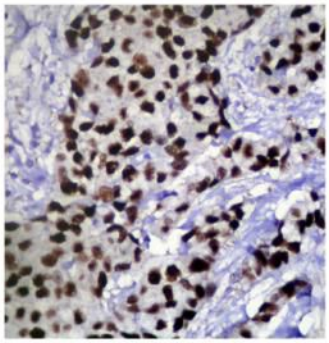
Optimal staining of cervix. The squamous and columnar cells show moderate to strong reaction.

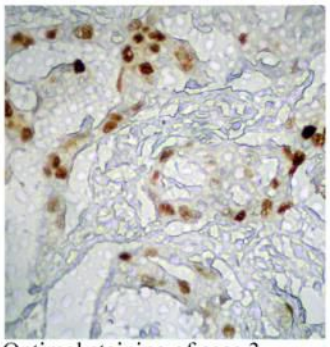


The left portion of the image shows non-staining of columnar cells. Post-menopausal cervix.

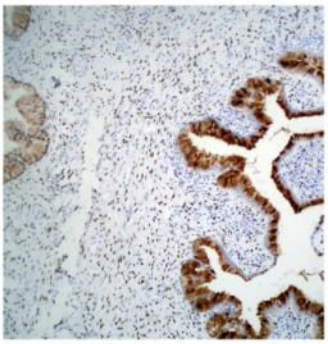


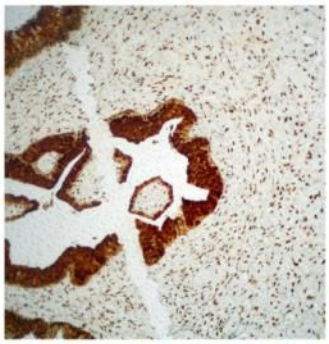
Optimal staining of case 4. Strong nuclear staining of the tumor cells.





Optimal staining of case 3. Weak to moderate staining is observed.





Cytoplasmic staining was observed with use of clone PgR636 from Dako. This did not affect interpretation of carcinoma cases, however.