Progesteron receptor (PR)

Cervix, positive 255/224 Med/Avg
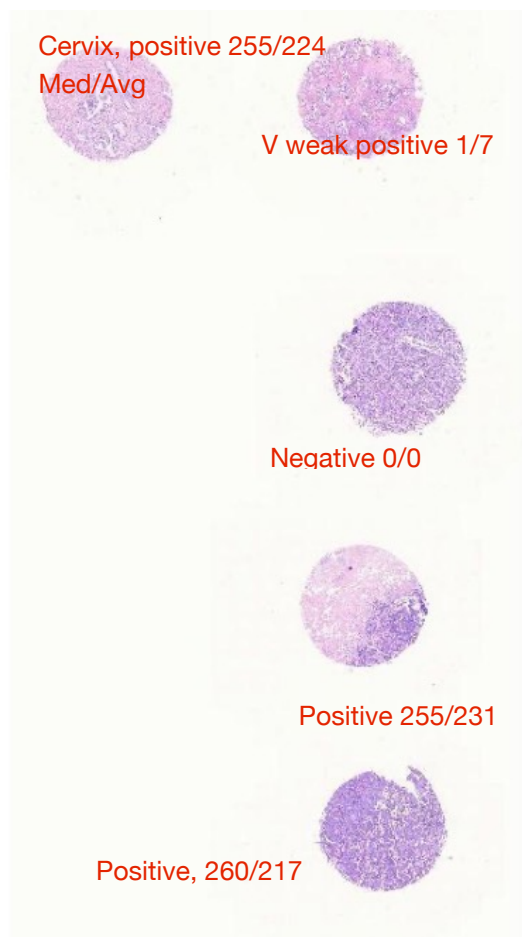
V weak positive 1/7

Negative 0/0

Positive 255/231

Positive, 260/217

HE staining in scanner view. The scores are median/average for each.

PSA immunostaining, scanner view

Provided sections in this run:

TISSUE	PR-POSITIVITY	PR-INTENSITY
Cervix	75%	Moderate to intense
Breast carcinoma	1% to 10%	Weak
Breast carcinoma	0%	Negative
Breast carcinoma	75%	Intense
Breast carcinoma	90%-100%	Intense

* PR-status and staining pattern as characterised by core participating laboratories using the rmAb clones pgr636 and 1E2

* All tissues were fixed in 10% neutral buffered formalin for 24-48 hours and processed according to Allison et al

Criteria for assessing staining as optimal included:

1. At least 60% moderate to intense staining of the cells in the cervix, whether stromal or columnar cells. This reflects as score more than 255 (median)
2. Staining in the appropriate proportion of cells in cases 4 and 5.
3. No nuclear staining in case 3.
4. At least score 1 in tissue 2 (very weak expression).
5. IxP score of each tissue should be more than or equal to the median of the same for the group.
6. Sum score (IxP for each tissue) should be more than median sum score of all participants.

Staining is considered good if:

1. The overall staining reaction is either weak in intensity or less in proportion,
2. Such situation would reflect as less than the median value for overall score, but has to be more than average to call it good staining. Since such a situation would also arise due to set of tissue with higher antigen getting well-stained while missing out on weaker staining cases. To avoid that, individual tissue score should also be above average to qualify.

Staining is considered borderline if:

1. The staining reaction is weak but the sum score of all tissue is still above the average sum score of all tissue in all participants.
2. However, the cervix has to stain at least to avoid the case being classified as poor.
3. All positive cases (i.e. case number 1,4 and 5) must get stained. Since expression of PR in tissue 2 is found to be extremely weak, we have ignored negative staining for borderline classification.

Staining is considered poor if:

1. The cervix fails to get stained.
2. Cases 4 or 5 fail to get stained.

Break up of results

Result	Participants
Optimum	14
Good	4
Borderline	10
Poor	10

Break up of results based on technique

Technique \ Result	Optimum	Good	Borderline	Poor	Total
Manual	9	2	4	6	21
Automated Ventana Bench Mark	1		2	1	4
Automated Ventana Bench Mark Ultra				1	1
Automated Ventana Bench Mark XT	1	1	2	2	6
Automated, Ventana Benchmark GX		1	1		2

Clones used in the run by participants

Clone	Participants
16	1
1E2	12
EP2	10
PgR636	5
QR014	1
SP2	5
ZR4	1

Break up of results based on the clones used

Result Clone	Optimum	Good	Borderline	Poor	Total
16	1				1
1E2	2	2	5	3	12
EP2	4	2	2	2	10
PgR636	2			3	5
QR014					1
SP2	2		2	1	5
ZR4				1	1

Break up of results based on the vendors

Result Clone	Optimum	Good	Borderline	Poor	Total
14/2041C				1	1
BIOCARE MEDICALS ACA 302 A			1		1
BIOCARE PRM302AA				1	1
BiogeneX AN711-5ME			1		1
Biogenex; QD400-60KEN	1				1
Dako				1	1
Dako, 11405263	1				1
Dako, IS068	1			1	2
DAKO, M356901				1	1
Diagnostic BioSystems, RMPD002			1		1
EPREDIA RM9102S0	1				1
Master diagnostic MAD -000670QD-R-3	1				1
PATH N SITU, CAT#HAR068				1	1
PathnSitu		1			1
PathnSitu and PR068					1
PathnSitu PR068	3	1	1	1	6
Quartett, P-P006-70					1
Thermo	1				1

Clone	Result	Optimum	Good	Borderline	Poor	Total
Ventana (240)05278392001-Roche#				1		1
Ventana (Catalogue No: H13339)					1	1
Ventana 790-4325		1				1
VENTANA BENCHMARK GX, H36443			1			1
Ventana, 790-4296		1	1	4	2	8

Break up of results based on format

Format	Result	Optimum	Good	Borderline	Poor	Total
RTU		10	4	8	9	33
Concentrated.		1		1	1	3

Break up of results based on temperature of dewaxing

Dewax Minutes	Result	Optimum	Good	Borderline	Poor	Total
>75				1	1	2
71-75		2	2	4	2	10
66-70			1	1	2	4
60-65		7	1	2	2	12
55-59		1		1	2	4
37					1	1
RT		1				1

Protocol used by the top 3 in this run

Break up of results based on pH of retrieval buffer

pH \ Result	Optimum	Good	Borderline	Poor	Total
6.0-6.9	2		1	2	5
7.0-8.0		1		2	3
8.1-9.0	8	3	7	6	24
>9.0	1				1

Break up of results based on incubation time of primary antibody

Time in minutes \ Result	Optimum	Good	Borderline	Poor	Total
15-30	2		5	4	11
31-45	2	1	1	4	8
46-60	7	3	2	2	14
>60			1		1

Turn overleaf for top 3 participants

VARIABLE	1	2	3
Technique	Automated, Ventana Benchmark GX	Manual	Automated Ventana Bench Mark
Clone	1E2	PgR636	1E2
Vendor	VENTANA BENCHMARK GX, H36443	Dako, 11405263	Ventana, 790-4296
Format	RTU	RTU	RTU
Batch/Year	7904296, 2022	Jul-22	BATCH NUMBER -H13339
Expiry	2024	Jul-23	11-05-2023
Dewaxing temperature	75 degree celcius	65° C	75*C
Retrieval	HIER	HIER	HIER
Enzyme	NA	N/A	NA
HIER	company system - cell conditioning 1 - CC1	PT link, DAKO	COMPANY SYSTEM -VENTANA BENCHMARK XT
Peak T and Time	95 degrees and 44 mins	97 degree centigrade,15 min	PEAK TEMPERATURE -100*C TIME- 30 MINUTES
Peak Pressure and Time	NA	N/A	NA
Retrieval Buffer	Tris EDTA buffer	DAKO, target retrieval buffer	COMPANY PROVIDED -CELL CONDITIONING(CC1)
pH	7.8	9	8.5
Blocking	3% Hydrogen peroxide	DAKO, 10 minutes	ULTRA VIEW INHIBITOR-4 MINUTES
Wash sol	TRIS based buffer solution	TRIS Buffer SALINE	REACTION BUFFER
Dilution of RTU	NO	No	10 x
Dilution of conc	NA	N/A	RTU
Diluent	NA	N/A	NA
Inc time of Primary	60 mins	1 hour	28 MIN
Detection	46-60	46-60	15-30
Cat No	Ultraview universal DAB detection kit	Polymer based	POLYMER BASED SYSTEM

| VARIABLE	1	2	3
Inc time of Sec	Ultraview universal DAB detection kit, Ventana benchmark GX,	Envision FLEX, high PH link,DAKO 41424210: Expiry- 31.03.2023	DETECTION SYSTEM -ULTRA VIEW COMPANY-ROCHE CATALOGUE NUMBER-760-500 BATCH NUMBER-H33655 EXPIRY-11-11-2023
Chro-substrate	16 mins	30 minutes	8 MINUTES
Post-treatment	8 mins	10 minutes	8 MINUTES
Counterstain	copper, 5 mins	No	COPPER SULPHATE-4 MINUTES
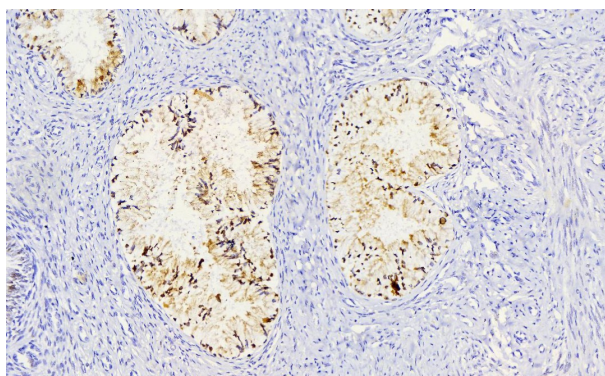
Cytoplasmic staining seen with clone pgr636. This should not affect the score. However, the intensity is weaker and stromal cells are unstained.
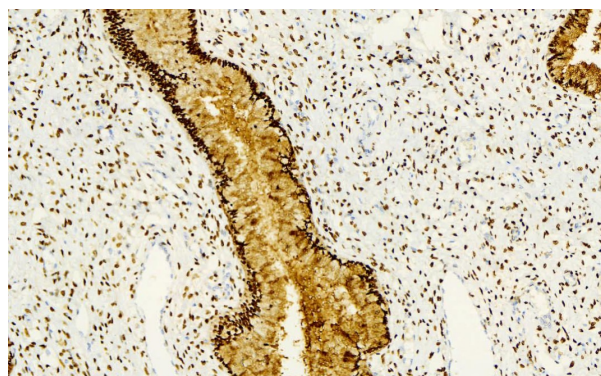
Optimal staining protocol that stains stromal and epithelial cells with good intensity and proportion. Cytoplasmic staining seen with clone pgr636.
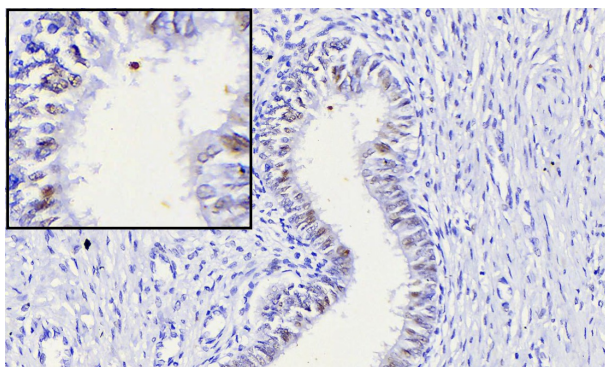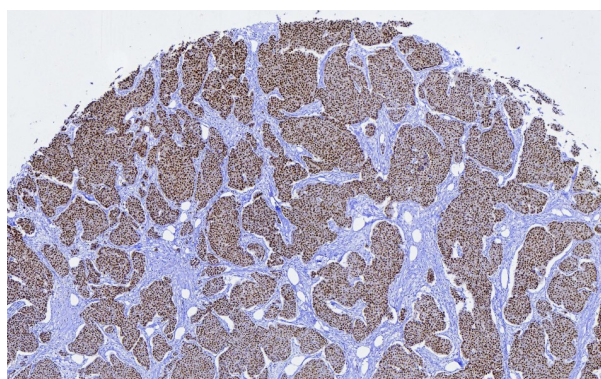

Rather weak nuclear staining of endocervical epithelial cells and no staining of stromal cells in participant having in appropriate epitope retrieval.
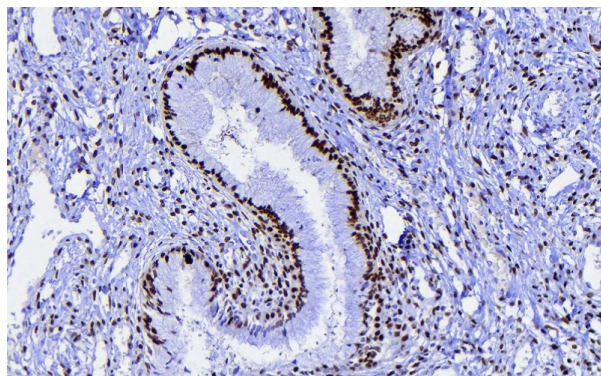
Optimum staining of epithelial and stromal cells of cervix by a participant.
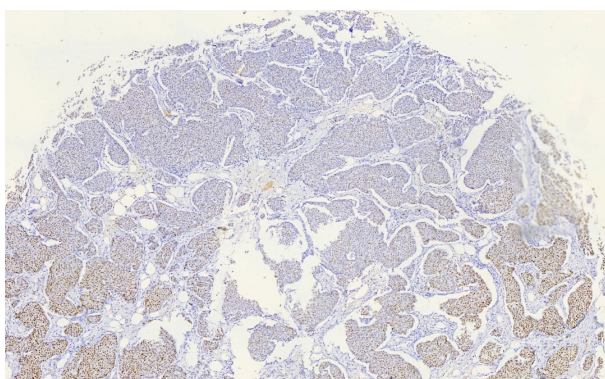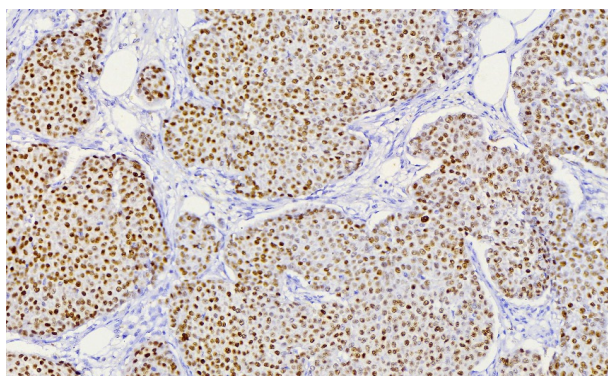

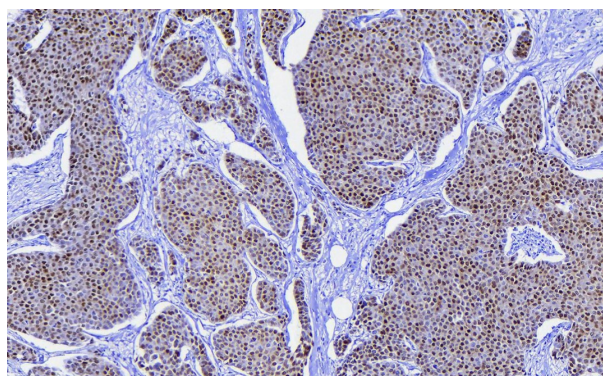*Case 5, effect of slant. The upper half of the image shows weaker staining.*

Case 5, Same case with optimal staining and no slant.

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Case 5, suboptimal staining using incompetent antibody

Case 5, suboptimal staining using optimum protocol