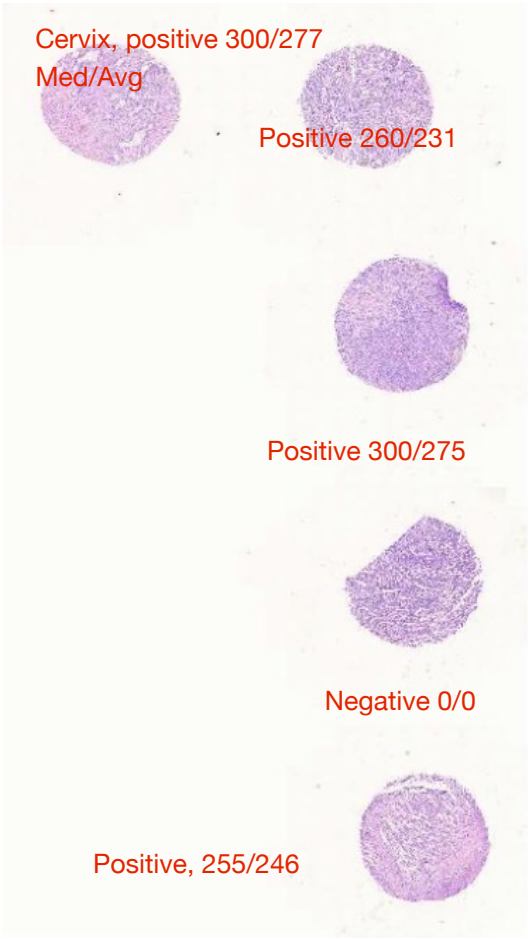
Estrogen receptor (ER)



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

PSA immunostaining, scanner view

Provided sections in this run:

TISSUE	ER-POSITIVITY	ER-INTENSITY
Cervix	60%	Moderate to intense
Breast carcinoma	80% to 90%	Moderate
Breast carcinoma	80% to 90%	Moderate to intense
Breast carcinoma	0%	Negative
Breast carcinoma	Tissue peeling anticipated. Remaining tissue should stain intensely in all cells.	Intense

* *ER-status and staining pattern as characterised by core participating laboratories using the rmAb clones EP1 and SP1*

* *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
2. Staining in the appropriate proportion of cells in cases 2,3 and 5.
3. No nuclear staining in case 4.
4. Tissue peeling is expected in case 5, however the remaining cells must show intense staining in all of them. Complete peeling of this tissue is not allowed.
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 so weak that it falls below the average score of individual tissue as well as for the entire set.
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 2,3 and 5) must get stained.

Staining is considered poor if:

1. The cervix fails to get stained.
2. Any of the positive cases fails to get stained.

In a situation where the tissue has peeled off, the case is assigned a remark.

Break up of results

Result	Participants
Optimum	20
Borderline	8
Poor	10

Break up of results based on technique

Technique \ Result	Optimum	Borderline	Poor	Total
Manual	12	4	6	23
Automated Ventana Bench Mark	3	3	4	10
AUTOMATED	1	1		2

Clones used in the run by participants

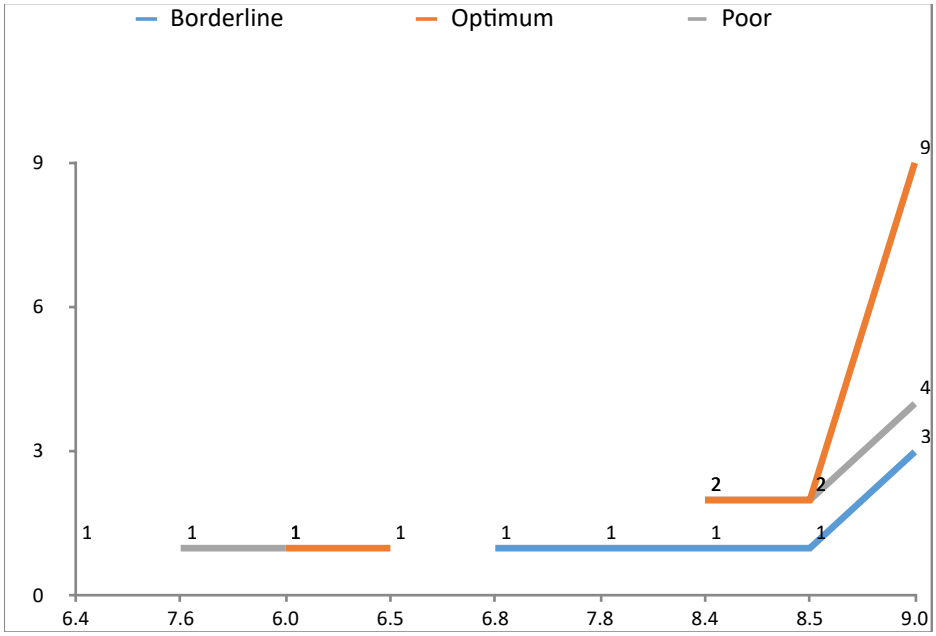
Clone	Participants
SP1	17
EP1	16
IHC 403	1
QR013	1

Break up of results based on the clones used

Clone \ Result	Optimum	Borderline	Poor	Total
SP1	8	4	4	17
EP1	8	4	4	16
IHC 403			1	1
QR013			1	1

Table 1-1

pH \ Result	Optimum	Borderline	Poor
6.0	1		1
6.4	1		
6.5	1		
6.8		1	
7.6			1
7.8		1	
8.4	2	1	2
8.5	2	1	2
9.0	9	3	4

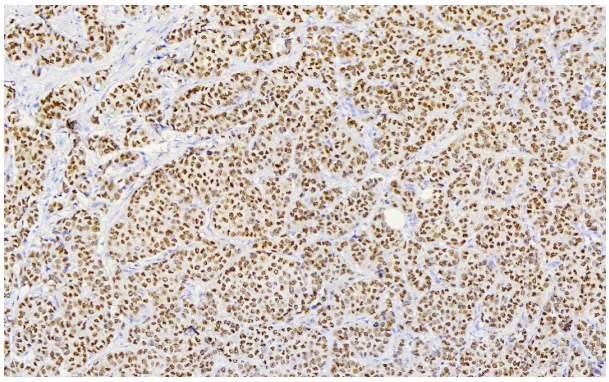


Protocol used by the top 3 in this run

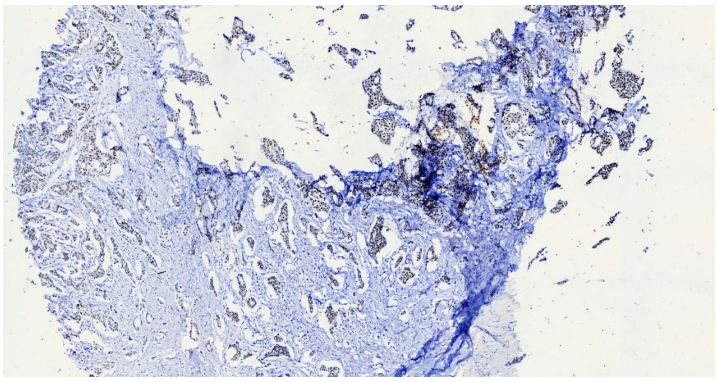
VARIABLE	1	2	3
Technique	Automated Ventana Bench Mark	AUTOMATED	Manual
Clone	SP1	SP1	SP1
Vendor	Ventana 790-4325	COMPANY-ROCHE CATALOGUE NUMBER-790-4325	Thermo
Format	RTU	RTU	Concentrated
Batch/Year	H20484 08-09-2021	BATCH NUMBER -H15308	Lot No- 9101S2107C
Expiry	02-09-2023	06-06-2023	7/2023
Dewaxing temperature	75° C	75° C	60° C
Retrieval	HIER	HIER	HIER
Enzyme	NA	NA	-
HIER	Company System (Ventana Bench Mark GX)	COMPANY SYSTEM -VENTANA BENCHMARK	pressure cooker
Peak T and Time	100 Degrees for 60 Minutes	PEAK TEMPERATURE -100*C TIME-30 MINUTES	100.C and 10 min.
Peak Pressure and Time	NA	NA	5 min.
Retrieval Buffer	Company Provided CC1 (Cell Condioner-1)	COMPANY PROVIDED -CELL CONDITIONING(CC1)	Retrieval buffer-inhouse made
pH	8.4	8.5	9.0
Blocking	Company provided Ultra view Universal DA H2O2 0.04% for4 Minutes	ULTRA VIEW INHIBITOR-4 MIN	N.A
Wash sol	Company provided Reaction Buffer	REACTION BUFFER	Tris buffered with twin 20
Dilution of RTU	No	10 x	-
Dilution of conc	NA	RTU	1:300
Diluent	NA	NA	AB Quanto, thermo
Inc time of Primary	16 Minutes	28 MIN	1 hour
Detection	Company Provided Ultra view Universal DAB Detection Kit	POLYMER BASED SYSTEM	polymer based system

General observations in Run 17

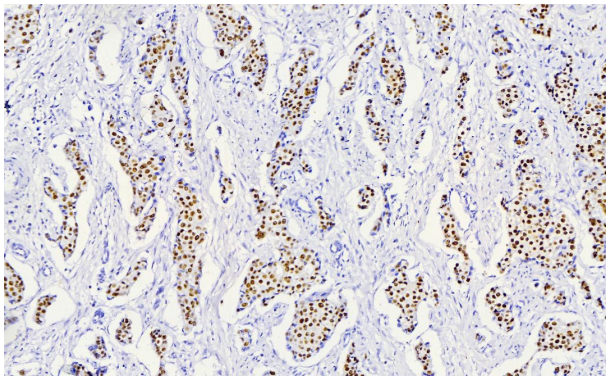
VARIABLE	1	2	3
Cat No	Ultra View Universal DAB Detection Kit Company- Ventana, Catalog Number 760-500, Batch Number- H33654, Manufacturing Date- 08-02-2022, Expiry date- 11-11-2023	DETECTION SYSTEM -ULTRA VIEW COMPANY-ROCHE CATALOGUE NUMBER-760-500 BATCH NUMBER-H33655 EXPIRY-11-11-2023	Polymer- Masterdiagnosis- 040322- 1/2024
Inc time of Sec	8 Minutes	8 MINUTES	30 min.
Chro-substrate	8 Minutes	8 MINUTES	5 min.
Post-treatment	Company Provided (Ultra view Universal DAB Copper) for 4 Minutes	COPPER SULPHATE-4 MINUTES	N.A
Counterstain	Company Provided Hematoxylin	HAEMATOXYLIN	4 deeps



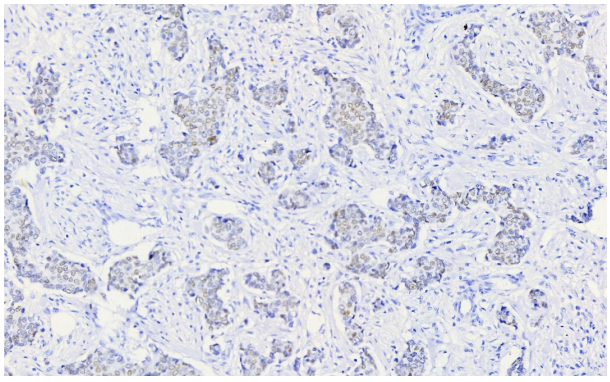
Case 3, optimal staining using clone EP1 at pH 9.2 using HIER with MWO



Peeling of tissue was noted more often in this run



Case 5, optimal staining using clone EP1 at pH 9.2 using HIER with MWO



Case 5, suboptimal staining using incompetent antibody