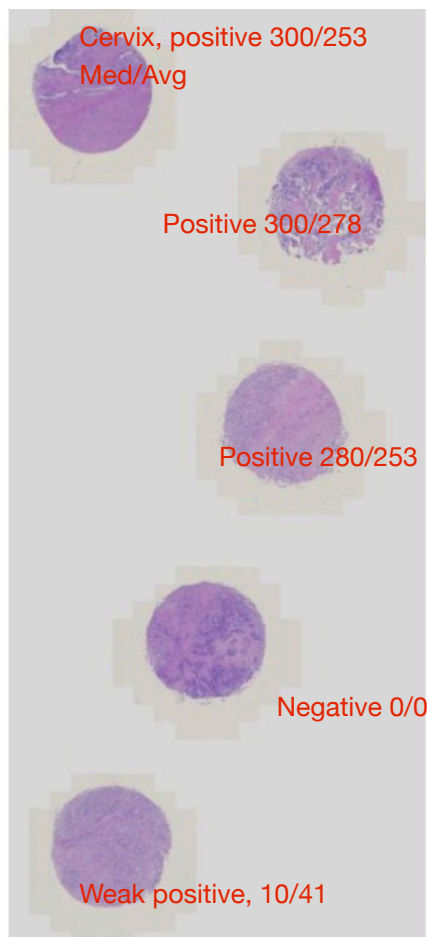
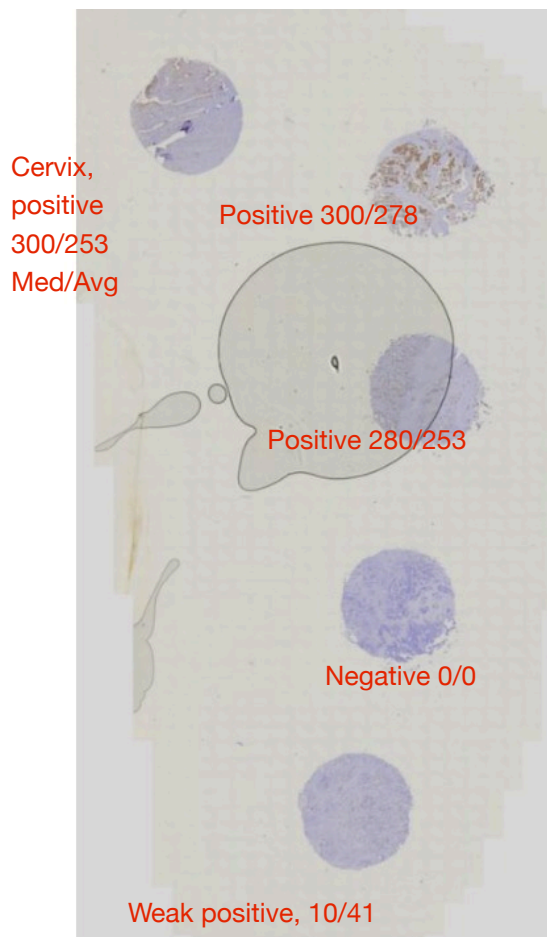




Estrogen receptor (ER)



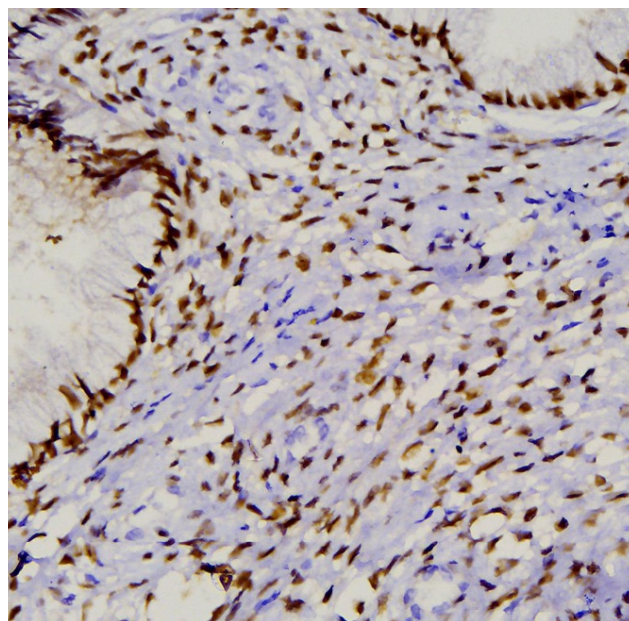
HE staining in scanner view. The scores are median/average for each.



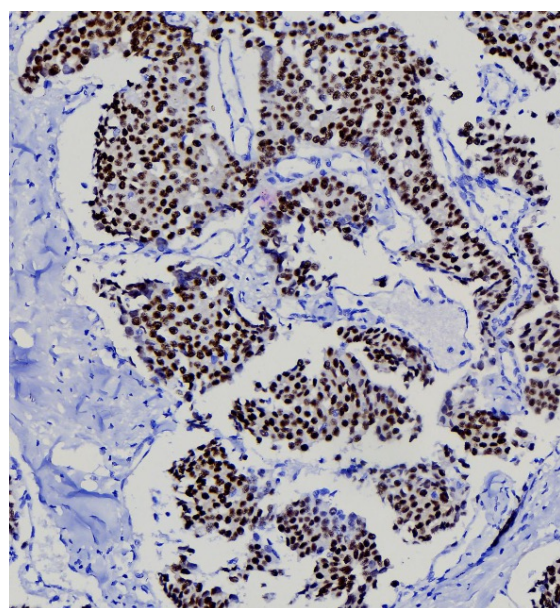
PSA immunostaining, scanner view

Provided sections in this run.

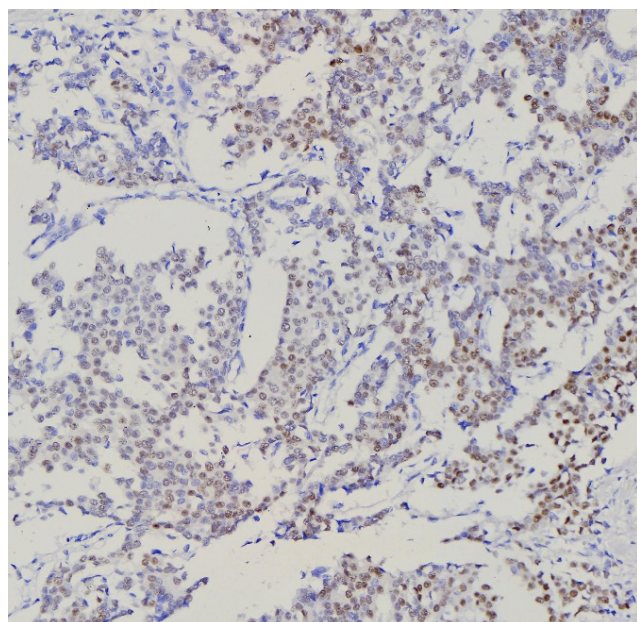
Tissue No. 5 is the most important tissue section in the theme. While cervix serves as a good positive control, many participants failed in the weak intensity case number 5. Since it is clinically most crucial to identify hormone receptor positivity, it would be imperative to have a right protocol setting that would identify the weakest of the lot.



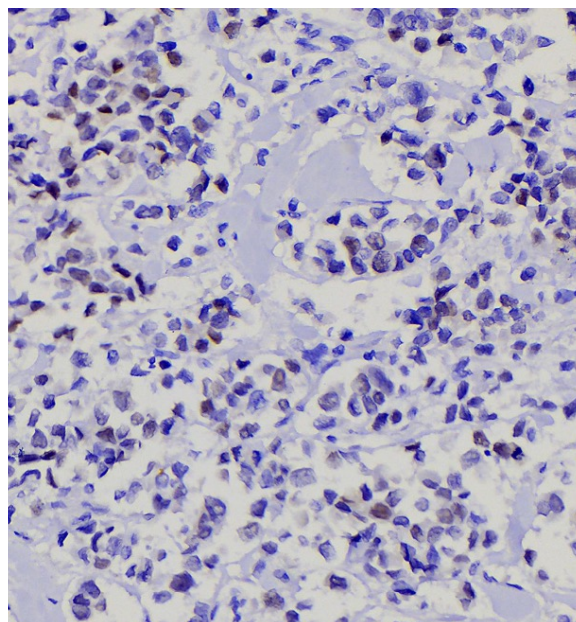
Intense nuclear staining of endocervical epithelial cells and the stromal cells



Optimum staining for ER in tissue number 2, in the participant who had cervix stained like the one on the left.



Suboptimal staining of tissue number 2 due to inadequate HIER



Optimum staining in case 5. Section from the core group.



Protocol used by the top 3 in this run

Variable	1	2	3
Technique	Automated Ventana Bench Mark	Manual	manual technique
Clone	SP1	EP-1	EP1
Vendor	Ventana 790-4325	Pathnsitu	PathnSitu catalogue number- PRO42
Format	RTU	RTU	RTU
Batch/Year	F23615 25-10-2019	R08042MA	Batch number- R08042OA year of manufacturing- 2020
Expiry	22-10-2021	06/2023	05/2023
Dewaxing temperature	75 Degree	65-68 degrees C	70 Degree Centigrade
Retrieval	HIER	HIER	HIER
Enzyme	NA	NA	NA
HIER	company system (Ventana Bench Mark GX)	Microwave oven	microwave oven
Peak T and Time	100 degrees for 60 minutes	900 watt, 15 minutes x 3 cycles	700 Microwave (20 minutes)
Peak Pressure and Time	NA	NA	NA
Retrieval Buffer	Company provided CC1 (Cell Conditioner-1)	In house made	Inhouse made
pH	8.4	8.0 to 9.0	9
Blocking	Company provided Ultra view Universal DA H2O2 0.04% for 4 minutes	Peroxidase block, prepared in house	30 percent hydrogen peroxide dilute into 1: 10 incubation time - 10 minutes
Wash sol	Company provided Reaction Buffer	Tris buffered saline with Tween 20	PBS (Phosphate buffered saline) without saponifying agent)
Dilution of RTU	No	No	NO
Dilution of conc	NA	NA	NA
Diluent	NA	NA	NA
Inc time of Primary	16 minutes	60 minutes	1 Hour

General observations in Run 16



Variable	1	2	3
Detection	Company provided Ultra view Universal DAB Detection Kit	Polymer based	polymer based system
Cat No	Ultra View Universal DAB Detection Kit Company - Ventana, Catalog Number 760-500 ,Batch number -H21101 , Manufacturing Date -20-10-2021 , Exp date- 14-7-2023	Peroxidase / DAB Dako Real Tm En Vision, K5007. Lot 20084627	Poly XL HRP/DAB Detection System, company - PathnSitu, OSHOO1-10 0ml, manufacturing date - 2021 expiry- 04/2023
Inc time of Sec	8 mintues	45 minutes	30 minutes
Chro-substrate	8minutes	3 to 5 minutes	10 minutes
Post-treatment	Company provided (Ultra view Universal DAB Copper) for 4 minutes	No	NO
Counterstain	Company provided Hematoxylin	Hematoxylin	Hematoxylin