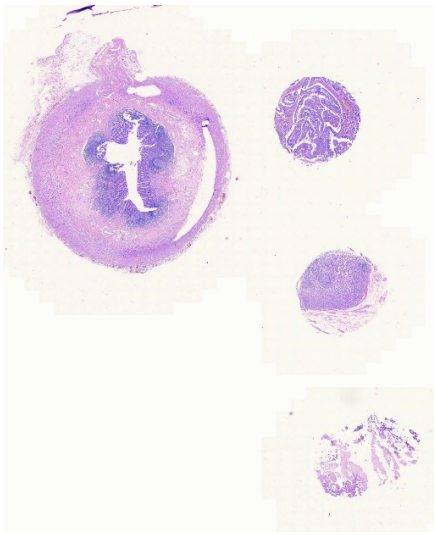
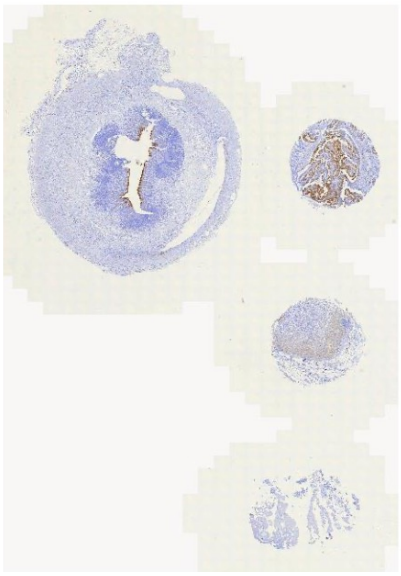
CK20



HE staining in scanner view.



Immunostaining, scanner view

Provided sections in this run:

TISSUE	PROPORTION	INTENSITY
Appendix	A strong, distinct cytoplasmic staining reaction of lining epithelial cells more towards the surface in the appendix and focal weak to moderate staining reaction in crypt cells.	Intense
Colonic adenocarcinoma	A weak to strong, distinct cytoplasmic staining reaction of the vast majority of neoplastic cells in the colon adenocarcinoma.	Intense
Stomach	Vast majority of the foveolar lining epithelial cells are unstained. Occasional focus of intestinal metaplasia shows moderate to intense cytoplasmic staining. This tissue showed non-specific staining in majority of the participant and hence removed from calculations. However, excessive background staining is factored in. The intestinal metaplasia is not represented in all participants and hence that is also discounted.	Weak
Urothelial carcinoma	Weak to moderate intensity staining in nearly 60% of the tumor cells.	Moderate

Performance characteristic of the group:

N	**35**
MEDIAN OVERALL SCORE	**50**
AVERAGE OVERALL SCORE	**40**

Criteria for assessment:

	Optimum	Good	Borderline	Poor
Overall score	>49	40-49	36-40	<36

Break up of results

Result	Participants
Optimum	**26**
Good	**3**
Borderline	**1**
Poor	**5**
Section not submitted	**9**

Break up of results based on technique

Result / Technique	Optimum	Good	Borderline	Poor	Total
Automated	1	0	0	1	**2**
Automated Ventana Bench Mark XT	4	0	0	2	**6**
Automated Ventana Benchmark Ultra	0	0	0	1	**1**
Automated, Ventana Benchmark GX	1	0	0	1	**2**
Automated, Ventana Medical systems, Inc., Roche Diagnostics	1	0	1	0	**2**
Manual	18	2	0	0	**20**
NA	1	1	0	0	**2**

Clones used in the run by participants

Clone	Participants
EP23	**8**
GM003	**1**
IT-KS208	**2**
Ks 20.8	**6**
Ks20.8	**10**
NA	**11**
SP33	**5**
ZM42	**1**

Break up of results based on the clones used

Clone / Result	Optimum	Good	Borderline	Poor	Section not submitted	Total
EP23	8	0	0	0	0	**8**
GM003	1	0	0	0	0	**1**
IT-KS208	1	0	0	1	0	**2**
Ks 20.8	3	1	0	1	1	**6**
Ks20.8	8	1	0	1	0	**10**
NA	2	1	0	0	8	**11**
SP33	2	0	1	2	0	**5**
ZM42	1	0	0	0	0	**1**

Break up of results based on the vendors

Vendor \ Result	Optimum	Good	Borderline	Poor	Total
Biocare	1	0	0	0	**1**
BIOCARE PM062A	1	0	0	0	**1**
BiogeneX AM315-5M	1	0	0	0	**1**
Biosystems, PDM049	1	0	0	0	**1**
Cell Mourque Cat 320-M-16-RUO	1	0	0	0	**1**
Company -Quartett catalogue number- KS20.8	1	0	0	0	**1**
Dako	1	0	0	0	**1**
DAKO IS777	3	1	0	1	**5**
DAKO M7019	1	0	0	1	**2**
Master Diagnostica; 005105QD-R-7	1	0	0	0	**1**
NA	1	1	0	0	**2**
PATH N SITU , CAT# HAR036	1	0	0	0	**1**
Pathnsitu	3	0	0	0	**3**
PathnSitu Catalogue No: MM1153	0	1	0	0	**1**
PathnSitu, PR036	5	0	0	0	**5**
quartett, P-C005-70	1	0	0	0	**1**
Ventana	1	0	0	0	**1**
Ventana (Catalogue No: AM3150321)	0	0	0	1	**1**
Ventana (240)05587760001	0	0	0	1	**1**
VENTANA 790-4431	1	0	1	1	**3**
Zeta corporation, 180	1	0	0	0	**1**

Break up of results based on format

Result / Format	Optimum	Good	Borderline	Poor	Section not submitted	Total
Concentrated	3	0	0	0	0	**3**
NA	1	1	0	0	8	**10**
RTU	22	2	1	5	1	**31**

Break up of results based on temperature of dewaxing

Result / Dewax Minutes	Optimum	Good	Borderline	Poor	Section not submitted	Total
37	1	0	0	0	0	**1**
>75	0	0	0	0	0	**0**
55-60	7	1	0	1	0	**9**
61-65	8	1	0	0	0	**9**
66-70	2	0	0	0	1	**3**
71-75	4	0	1	3	0	**8**
NA	1	1	0	0	8	**10**
RT	1	0	0	0	0	**1**

Break up of results based on pH of retrieval buffer

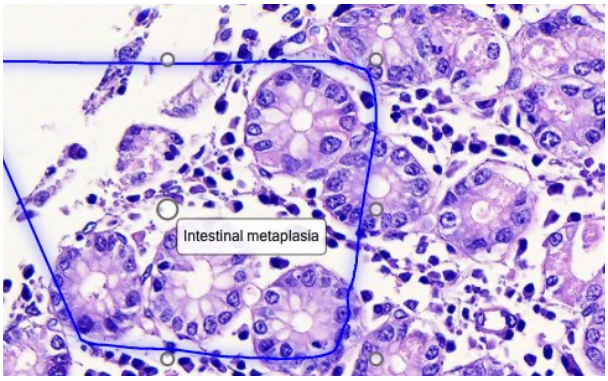
Result / pH	Optimum	Good	Borderline	Poor	Section not submitted	Total
6.0-6.9	2	1	0	0	0	**3**
7.0-8.0	3	0	0	1	0	**4**
8.1-9.0	17	1	1	4	1	**23**
>9.0	0	0	0	0	0	**0**
NA	2	1	0	0	8	**3**

Break up of results based on incubation time of primary antibody

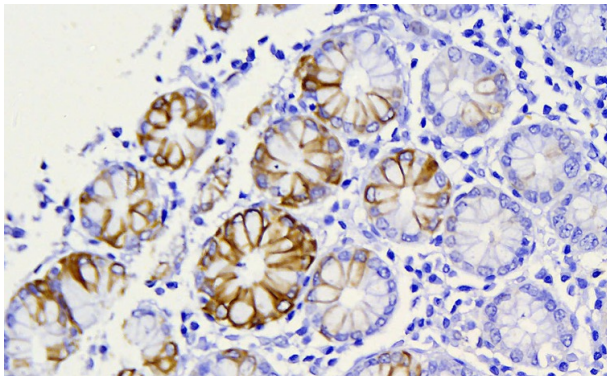
Result / Time in minutes	Optimum	Good	Borderline	Poor	Section not submitted	Total
<15	0	0	0	1	0	**1**
15-30	6	0	0	2	0	**8**
31-45	7	0	1	2	0	**10**
46-60	12	2	0	0	1	**15**
NA	2	1	0	0	8	**11**

General observations in Run 17

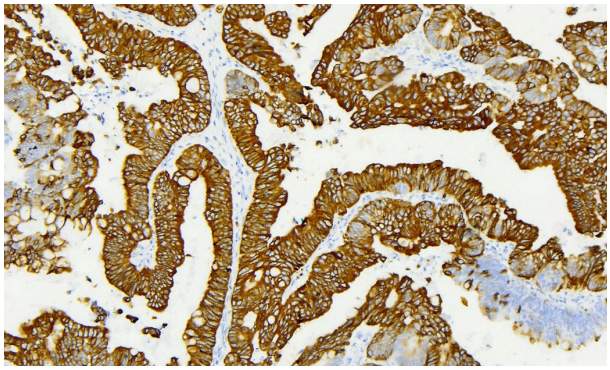
VARIABLE	1	2	3
Technique	Automated, Ventana Benchmark GX	Manual Technique	Manual
Clone	SP33	Ks20.8	GM003
Vendor	VENTANA BENCHMARK GX , H09036NA	DAKO IS777	PathnSitu, catalogue number: PR036
Format	RTU	RTU	RTU
Batch/Year	HAR036,2022	20082795	R08036MA, 2020
Expiry	2023	19/05/2022; validation done and results are satisfactory	06/23
Dewaxing temperature	75 degree celcius	65 degree	58-60°C
Retrieval	HIER	HIER EDTA Buffer pH 9.0	HIER
Enzyme	NA	---	NA
HIER	company system - cell conditioning 1 - CC1	Micro wave	Pressure cooker
Peak T and Time	95 degrees and 44 mins	98 degree 20 minutes	NA
Peak Pressure and Time	NA	98 degree 20 minutes	Pressure cooker, 3 whistles, 5 min
Retrieval Buffer	Tris EDTA buffer	In house	In house made
pH	7.8	EDTA Buffer pH 9.0	pH:9
Blocking	3% Hydrogen peroxide	3 % Hydrogen peroxide with methanol 15 minutes	10-12 minutes
Wash sol	TRIS based buffer solution	TBST buffer pH 7.4	PBS Wash solution
Dilution of RTU	NO	---	NO
Dilution of conc	NA	---	RTU
Diluent	NA	0	NA
Inc time of Primary	60 mins	30 minutes	1 hour
Detection	Ultraview universal DAB detection kit	Polymer based system	Poly Excel HRP/DAB Detection system
Cat No	Ultraview universal DAB detection kit, Ventana	Master Polymer plus detection system (Peroxidase)	Poly Excel HRP/DAB Detection system, Pathnsitu
Inc time of Sec	16 mins	30 minutes	15 minutes
Chro-substrate	8 mins	4-6 minutes	3-5 minutes
Post-treatment	copper, 5 mins	---	No
Counterstain	Hematoxylin	Hematoxylin	Hematoxylin



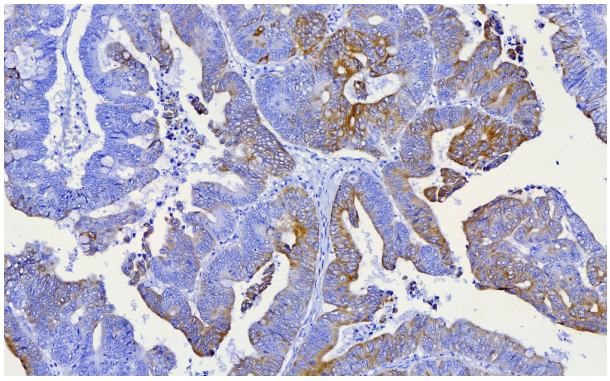
Focus of intestinal metaplasia in gastric biopsy



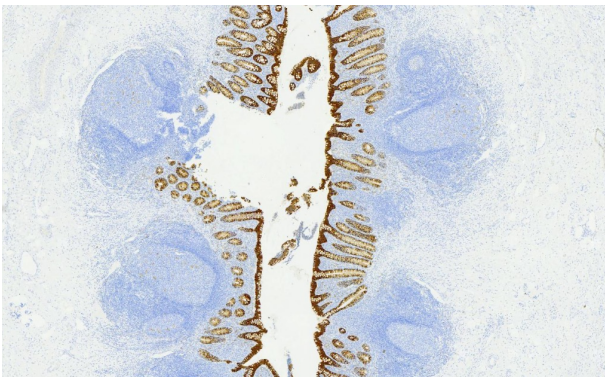
The focus stains with CK20. However, many participants failed to show this up, may be due to non-representation of the focus in their sections.



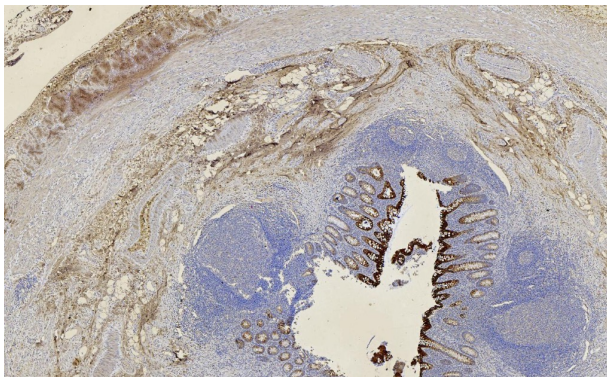
Optimum staining of colonic adenocarcinoma



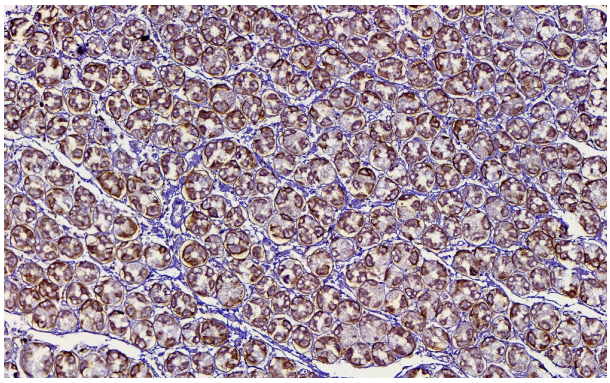
Suboptimal staining of colonic adenocarcinoma due to inadequate epitope retrieval.



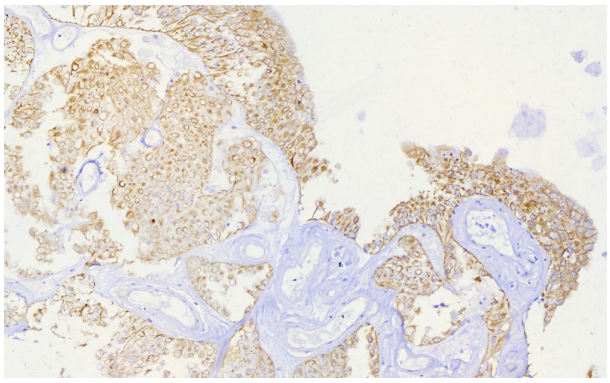
Optimum staining of appendicular crypts. The staining is more pronounced towards surface while the basal crypts also stain with moderate intensity. There is no staining of any other cell.



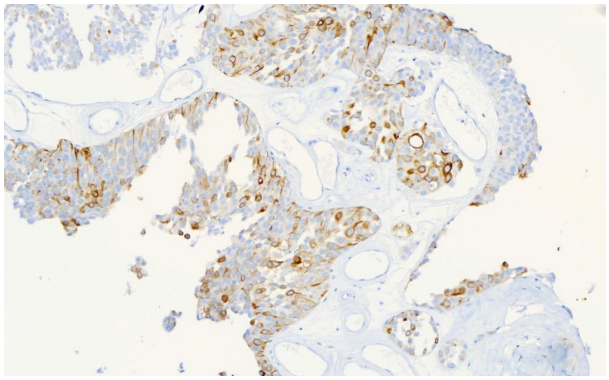
Suboptimal protocol staining has resulted in huge background staining and non-specific staining of connective tissue cells



Non-specific staining of gastric mucosa due to inappropriate protocol setting.



Optimum staining of urothelial carcinoma.



Optimum staining of urothelial carcinoma