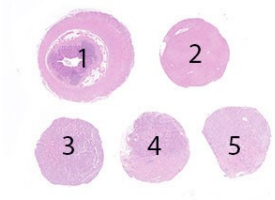
Assessment Run 74 2025

E-Cadherin (ECAD)

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of the IHC assays for ECAD performed by the NordiQC participants for the differentiation between breast lobular carcinoma and ductal carcinoma.
Relevant clinical tissues, both normal and neoplastic, were selected to include a wide spectrum of ECAD antigen densities (see below).

Material
The slide to be stained for ECAD comprised:



1. Appendix, 2. Liver, 3. Ductal breast carcinomas, 4-5. Lobular breast carcinoma.

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing an ECAD staining as optimal included:

- A strong, distinct membranous staining reaction of the epithelial cells of the bile ducts and an at least moderate membranous staining reaction of virtually all hepatocytes in the liver.
- A strong, distinct membranous staining reaction of virtually all the columnar epithelial cells in the appendix.
- A moderate to strong, distinct membranous staining reaction of virtually all neoplastic cells of the breast ductal carcinoma.
- No staining reaction or at maximum a focal and weak membranous staining reaction of the neoplastic cells of the breast lobular carcinomas.
- No staining reaction of stromal cells e.g. lymphocytes and plasma cells in lamina propria of the colon mucosa.

KEY POINTS FOR ECAD IMMUNOASSAYS
- The mAb clone **NCH-38** is recommendable both as a concentrated Ab and an RTU.
- The mAb clone **36** is recommendable as an RTU.
- Efficient HIER in an alkaline buffer is important for an optimal performance.
- The rmAb clone **EP700Y** and mAb clone **36B5** were less successful.

Participation

Number of laboratories registered for ECAD, run 74	460
Number of laboratories returning slides	430 (93%)

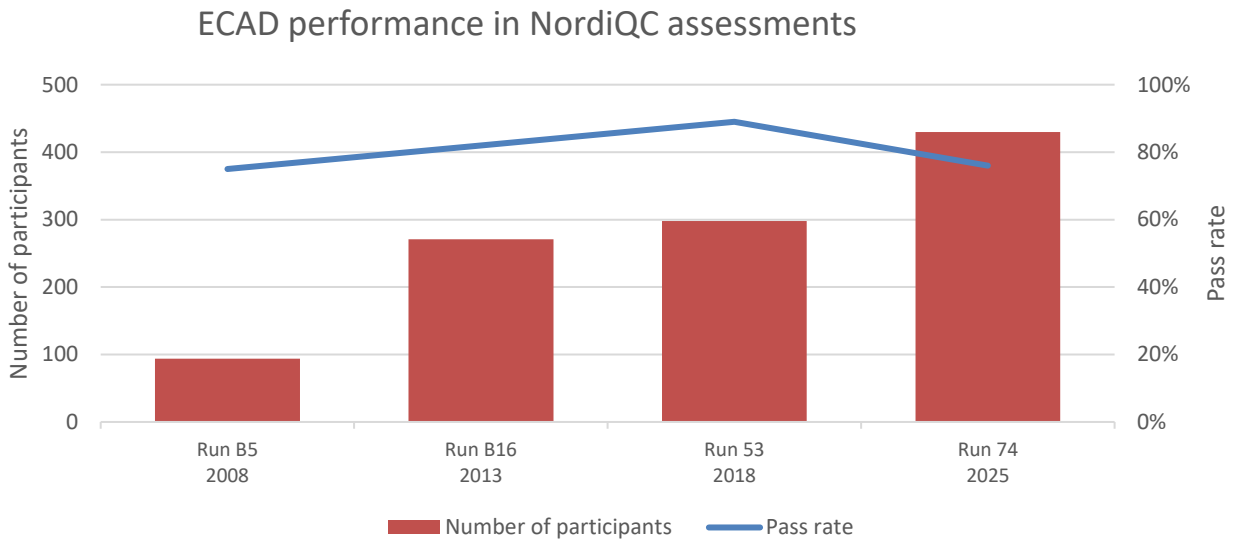
All slides returned after the assessment were assessed and received advice if the result being insufficient, but the data were not included in this report.

Results
430 laboratories participated in this assessment. Of these, 325 (76%) achieved a sufficient mark (optimal or good) – see Table 1a (page 3). Tables 1b and 1c summarizes the antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining reactions were:
- Inefficient HIER e.g., too short HIER time
- Poor signal-to-noise ratio of assays based on the rmAb EP700Y and mAb 36B5
- Use of less successful primary Abs

Performance history
This was the fourth NordiQC assessment of ECAD. A decrease in pass rate was seen compared to run 53, 2018 (see Graph 1). The number of participants has increased significantly in this run, and first-time-participants obtained a pass rate of 70%, 53% optimal, compared to 80%, 61% optimal, obtained by laboratories also participating in previous run 53.

Graph 1. **Proportion of sufficient results for ECAD in the four NordiQC runs performed**



Controls
Liver and appendix/colon are recommended as positive and negative tissue controls for ECAD. In liver, the protocol must be calibrated to provide an at least moderate, distinct membranous staining reaction of virtually all hepatocytes. Epithelium of the bile ducts should display a strong and distinct membranous staining intensity.
In the appendix/colon, only epithelium should display a strong membranous staining reaction. No staining reaction must be seen in stromal cells such as lymphocytes, plasma cells, smooth muscle cells or endothelial cells.

Conclusion
The mouse monoclonal Ab (mAb) clones **NCH-38** and **36** could be used to obtain optimal staining results for ECAD. Irrespective of the clone applied, efficient HIER, preferable in an alkaline buffer, and careful calibration of the primary Ab were the most important prerequisites for an optimal staining result. The most common Ready-To-Use (RTU) systems (IS/IR/GA059 and 790-4497) based on the mAb clones NCH-38 (Dako/Agilent) and 36 (Ventana/Roche), respectively, provided the highest proportion of sufficient and optimal results. The RTU system GA059 (Omnis) was very robust, giving a pass rate of 98% (92 of 94). Assays based on the mAb clone **36B5** and rmAb clone **EP700Y**, both as concentrated (Conc) formats and RTU systems, were challenged by excessive background and cytoplasmic staining hampering interpretation of the specific signal for ECAD.

Table 1a. **Overall results for ECAD, run 74**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	96	40	26	25	5	69%	42%
Ready-To-Use antibodies	334	208	51	70	5	77%	62%
Total	430	248	77	95	10		
Proportion		58%	18%	22%	2%	76%	

1) Proportion of sufficient stains (optimal or good).
2) Proportion of Optimal Results.

Table 1b. **Concentrated antibodies and assessment marks for ECAD, run 74**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **NCH-38**	68	Dako/Agilent	38	24	6	0	91%	56%
mAb clone **36B5**	8	Leica Biosystems	1	1	4	2	25%	13%
mAb clone **4A2C7**	1	Invitrogen	1	0	0	0	-	-
mAb clone **BS38**	1	Nordic Biosite	0	0	1	0	-	-
mAb clone **ECH-6**	2	Zytomed Systems	0	0	2	0	-	-
mAb clone **HECD-1**	1	Invitrogen	0	0	0	1	-	-
mAb clone **ZM63**	1	Zeta Corporation	0	0	1	0	-	-
mAb clone **IHC564**	1	GenomeMe	0	1	0	0	-	-
rmAb clone **EP700Y**	7	Cell Marque	0	0	7	0	-	-
rmAb clone **EP6**	1	Biocare Medical	0	0	1	0	-	-
	1	Diagnostic Biosystems	0	0	0	1	-	-
	1	BIO SB	0	0	1	0	-	-
rmAb clone **ZR375**	1	Zeta Corporation	0	0	0	1	-	-
Ab clone **4A2C7**	1	Wondfo	0	0	1	0	-	-
Ab clone **HGL-ECAD**	1	Bio-Highgrade	0	0	1	0	-	-
Total	96		40	26	25	5		
Proportion			42%	27%	26%	5%	69%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).

Table 1c. **Ready-To-Use antibodies and assessment marks for ECAD, run 74**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **36 790-4497 (VRPS)[3]**	34	Roche/Ventana	25	8	1	0	97%	71%
mAb clone **36 790-4497 (LMPS)[4]**	105	Roche/Ventana	64	28	11	2	88%	61%
mAb clone **ZM63 8269-C010**	3	Sakura Finetek	3	0	0	0	-	-
mAb clone **NCH-38 GA059 (VRPS)[3]**	61	Dako/Agilent	56	3	1	1	97%	92%
mAb clone **NCH-38 GA059 (LMPS)[4]**	39	Dako/Agilent	36	3	0	0	100%	92%
mAb clone **NCH-38 IS/IR059 (VRPS)[3]**	11	Dako/Agilent	9	2	0	0	100%	82%
mAb clone **NCH-38 IS/IR059 (LMPS)[4]**	13	Dako/Agilent	9	3	1	0	92%	69%
mAb clone **MX020 MAB-0738**	1	Fuzhou Maixin	0	1	0	0	-	-
mAb clone **SPM471 PDM182**	1	Diagnostic Biosystems	1	0	0	0	-	-
mAb clone **HECD-1 MAD-000761QD**	1	Master Diagnostica	0	0	1	0	-	-
mAb clone **35B5 PA0387 (VRPS)[3]**	22	Leica Biosystems	0	1	21	0	5%	0%
mAb clone **35B5 PA0387 (LMPS)[4]**	9	Leica Biosystems	0	0	9	0	0%	0%
mAb clone **EP6 API3012**	1	Biocare Medical	0	0	1	0	-	-
mAb clone **IHC564 IHC564**	1	GenomeMe	0	1	0	0	-	-
rmAb clone **EP700Y 760-4440 (VRPS)[3]**	7	Roche/Ventana	0	0	7	0	0%	0%
rmAb clone **EP700Y 760-4440 (LMPS)[4]**	19	Roche/Ventana	2	1	15	1	16%	11%
rmAb clone **EP700Y 246R-18**	2	Cell Marque	0	0	1	1	-	-
Ab clone **499C4F1 PA073**	1	Abcarta	1	0	0	0	-	-
Ab clone **BY149 BFM-0155**	1	Bioin Biotechnology	1	0	0	0	-	-
Ab clone **C12A15 CER-0022**	1	Celnovte	1	0	0	0	-	-
Ab clone **GR111 GT2107**	1	Gene Tech	0	0	1	0	-	-
Total	334		208	51	70	5		
Proportion			62%	15%	21%	2%	77%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 assessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 assessed protocols).

Detailed analysis of ECAD, Run 74
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **NCH-38**: Protocols with optimal results were all based on Heat Induced Epitope Retrieval (HIER) using Bond Epitope Retrieval Solution 2 (BERS2; Leica Biosystems) (7/16)*, Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (9/10) or Cell Conditioning 1 (CC1; Ventana/Roche) (22/40) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings, 62 of 67 (93%) laboratories produced a sufficient staining result.
* (number of optimal results/number of laboratories using this HIER buffer)

Table 2. **Proportion of optimal results for ECAD for the most commonly used antibody as concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond[3]**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
mAb clone **NCH-38**	¾**	-	6/6 (100%)	-	22/40 (55%)	-	7/16 (44%)	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective syatems.
** (number of optimal results/number of laboratories using this buffer)
1) Autostainer Classical, Link 48.
2) BenchMark GX, XT, Ultra, Ultra plus
3) Bond III, Prime

Ready-To-Use antibodies and corresponding systems
mAb clone **36**, product no. **790-4497**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-64 min. at 95-100°C), 12-32 min. incubation of the primary Ab. And UltraView (760-500) or OptiView (760-700) as detection systems. Using these protocol settings 125 of 137 (91%) laboratories produced a sufficient staining result.

rmAb clone **EP700Y**, product no. **760-4440**, Ventana/Roche, BenchMark Ultra/Ultra Plus:
Protocols with optimal results were based on HIER using CC1 (efficient heating time 64 min. at 95°C), 24 min. incubation of the primary Ab. And UltraView (760-500) as detection system. Using these protocol settings 2 of 3 laboratories produced a sufficient staining result.

mAb clone **NCH-38**, product no. **IS/IR069**, Dako/Agilent, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 95-99°C), 20-30 min. incubation of the primary Ab and EnVision FLEX+ (K8000/K8002) as detection system. Using these protocol settings, 16 of 16 (100%) laboratories produced a sufficient staining result.

mAb clone **NCH-38**, product no. **GA059**, Dako/Agilent, OMNIS:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 20-30 min. at 97°C), 20-30 min. incubation of the primary Ab and EnVision FLEX+ (GV800/GV823+GV821) as detection system. Using these protocol settings, 92 of 94 (98%) laboratories produced an optimal staining.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 assessed protocols). The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included (in Table 1 LMPS also includes off label use on deviant IHC stainers).

Table 3. **Proportion of sufficient and optimal results for ECAD for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb NCH-38 **IS/IR059**	100% (11/11)	82% (9/11)	83% (5/6)	67% (4/6)
Dako Omnis mAb NCH-38 **GA059**	97% (59/61)	92% (56/61)	100% (33/33)	94% (31/33)
VMS XT/GX/Ultra/Ultra Plus mAb 36 **790-4497**	97% (33/34)	74% (25/34)	88% (91/103)	62% (64/103)
VMS Ultra/Ultra Plus rmAb EP700Y **760-4440**	0/7 (0%)	0/7 (0%)	3/19 (16%)	2/19 (11%)
Leica Bond III/Prime mAb 36B5 **PA0387**	1/22 (5%)	0/22 (0%)	0/8 (0%)	0/8 (0%)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Significant modifications: retrieval method, retrieval duration and Ab incubation time altered >25%, detection kit – only protocols performed on the specified vendor IHC stainer were included.

Comments

In this fourth NordiQC assessment for ECAD, the prevalent features of an insufficient staining result were characterized either by a poor-signal-to noise ratio or excessive background hampering the read-out of ECAD and primarily caused by the use of the rmAb clone EP700Y or mAb clone 36B5, seen in 73% of the insufficient results (77 of 105). The background reaction was both seen in the diagnostic samples and control tissues and illustrated in Figs. 1b - 4b. As seen in Figs. 1b-4b, the rmAb clone EP700Y typically gave a general background reaction in many cell types and tissues, while mAb clone 36B5 in addition gave an extended cytoplasmic staining reaction in the ductal carcinoma challenging the identification of true membrane reaction (see Fig. 7b). Too weak or false negative staining result was observed in 27% of the insufficient results (28 of 105). Virtually all laboratories were able to demonstrate ECAD in high-level antigen expressing cells, e.g., normal columnar cells of the appendix and epithelial cells of the bile ducts in the liver. However, the assays must be carefully calibrated according to low-level antigen expressing cells as normal hepatocytes showing an at least moderate distinct membranous staining intensity. Otherwise, and from a diagnostic point of view, it may be difficult to discriminate between ductal breast carcinoma displaying a weak or focal membranous staining reaction (the neoplastic cells should normally show a strong, complete and distinct membranous staining intensity) from lobular breast carcinoma in which the neoplastic cells totally lack or often also displays a weak focal membranous staining reaction.

22% (96 of 430) of the laboratories used a LD-assay for detection of ECAD. The mAb clone **NCH-38** was by far the most commonly used primary Ab within a LD-assay and provided a pass rate of 91% (62 of 68) of which 56% (38 of 68) were assessed as optimal (see Table 1b). The vast majority successfully used a 3-step detection system, giving a pass rate of 98% (53 of 54), 61% optimal (n=33). If using a 2-step detection system a lower pass rate of 64% (9 of 14) was obtained, 36% optimal (n=5). As shown in Table 2, the mAb clone NCH-38 could provide optimal results on all main IHC platforms from the three major vendors. The main causes for insufficient results were use of HIER in acidic buffer and use of a less sensitive detection system.

The mAb clone **36B5** provided an inferior pass rate of 25% (2 of 8), compared to last assessment run 53, where a high pass rate of 92% (12 of 13) was seen. Less distinct membranous staining reaction of cellular structures expected to be demonstrated together with background staining, was the main causes for the overall decrease in performance. No technical parameters could be identified, as similar protocol settings were applied as for mAb clone NCH-38.

78% (334 of 430) of the laboratories used a RTU system for detection of ECAD. In this assessment, the RTU systems IS/IR/GA059 (Dako/Agilent) and 790-4497 (Ventana/Roche) based on the mAb clones NCH-38 and 36, respectively, provided high pass rates and proportion of optimal results (see Table 1b).

For the RTU system **GA059** on the Omnis (Dako/Agilent), an overall pass rate of 98% (92 of 94) was seen. Both vendor and laboratory modified protocol settings (typically minor adjustments in HIER, incubation time of the primary Ab and/or choice of detection system) could be used to obtain optimal result (see Table 3).

The RTU system **IS/IR059** (Dako/Agilent) on the Autostainer provided a pass rate of 100% (11 of 11) when using the recommended protocol settings, 82% optimal (see Table 3). If modifying the protocol, a pass rate of 83% (5 of 6) was seen. The one insufficient result was caused be diluting the RTU Ab.

The RTU system **790-4497** based on the mAb clone 36 (Ventana/Roche), provided a pass rate of 97% (33 of 34) when following the recommended protocol settings, based on HIER in CC1 for 64 min., 24 min. incubation of primary Ab and UltraView as detection system. The majority of participants modified the protocol. Successful modifications were prolonging incubation time of primary Ab to e.g. 32 min. or use of OptiView as detection system. In general, a pass rate of 92% was obtained when using either OptiView or Ultra View with amplification as detection system (61 of 66), 73% optimal (n=48). A similar pass rate of 89% (63 of 71) was seen when using UltraView, but fewer optimal results was seen; 58% (n=41). Efficient HIER for 32-64 min. depending on the detection system (mean HIER for optimal results were 60 min. with UltraView and 48 min. with OptiView), seems mandatory to obtain sufficient results, as 92% (12 of 13) of the insufficient protocols, were based on HIER ≤36 min.

The RTU system **760-4440** based on the rmAb clone EP700Y (Ventana/Roche), provided a pass rate of 0% using the recommended protocol settings, based on HIER in CC1 for 36 min., 16 min. incubation of the primary Ab and UltraView as detection system. Six participants performed HIER for 64 min., and prolonged incubation time of primary Ab (24-32 min.) of which 3 obtained a sufficient result. Excessive background and cytoplasmic staining reaction were the prevalent features of an insufficient result. The same problem has been observed in previous runs (B16, 2013 and run 53, 2018). It is strongly recommended for participants using this assay to change to a more specific primary Ab as mAb clone 36, prod id. 790-4497 with same intended use and IHC stainer platform (see above). Surprisingly, the lobular breast carcinoma provided in most cases the expected optimal reaction pattern and from a diagnostic/clinical point of view, the assay in this assessment could be used to distinguish between ductal and lobular breast carcinomas. However, the excessive background staining in cellular structures expected to be negative (all other cores assessed), accounted for the overall insufficient performance (see Figs. 1b-4b).

Similar inferior results were observed for the RTU system **PA0387** for Bond (Leica Biosystems) based on mAb clone 36B5. An overall pass rate of 3% (1 of 30) was seen which is significantly lower compared to last run 53 (2018), with a 100% pass rate (6 of 6), thus no optimal. All insufficient results were caused by poor signal-to-noise ratio, excessive background and/or cytoplasmic staining reaction. No plausible explanation for the relatively significantly inferior performance of both the concentrated format and the corresponding RTU format of mAb clone 36B5 can be identified from the protocol settings applied in this assessment compared to previous assessments. However, in all previous assessments the clone in general provided a very low level of optimal results (less than 10% overall for RTU and Conc.) and the selection of tissues in this run might have revealed this inappropriate reaction pattern for the Ab. For laboratories having problems as observed in this run with the Ab, a change to another clone should be considered.

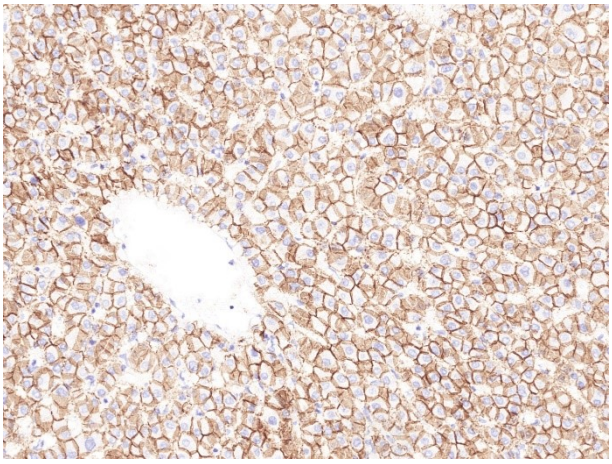


Fig. 1a
Optimal staining reaction of ECAD in the liver using the RTU system (GA059/Omnis, Dako/Agilent) based on the mAb clone NCH-38, HIER in TRS High pH and EnVision Flex+ as detection system. The hepatocytes display a moderate, distinct membranous staining reaction.
Same protocol used in Figs. 2a – 5a.

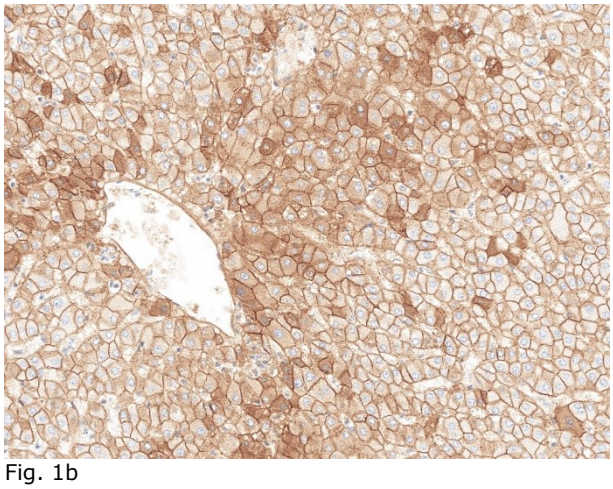


Fig. 1b
Staining reaction of ECAD in the liver using the RTU format (760-4440, Ventana/Roche) based on rmAb EP700Y on a Benchmark platform, HIER performed in CC1 and UltraView used as detection system – same field as in Fig. 1a.
Note a more extended cytoplasmic staining reaction in hepatocytes at the portal room is observed.
Same protocol used in Figs. 2b – 4b.

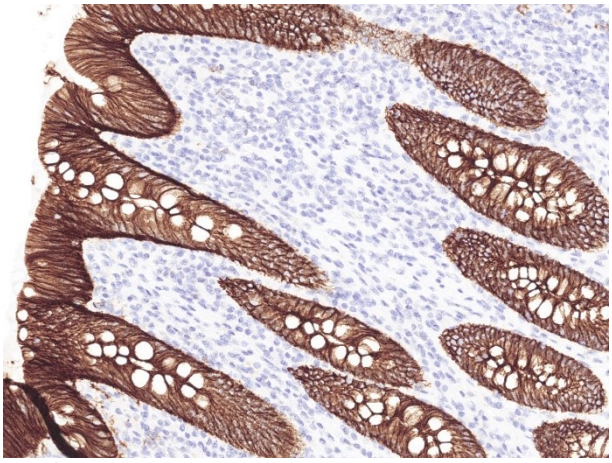


Fig. 2a
Optimal ECAD staining reaction of the appendix using same protocol as in Fig. 1a. The columnar epithelial cells display a strong membranous staining reaction while the stromal cells are negative.

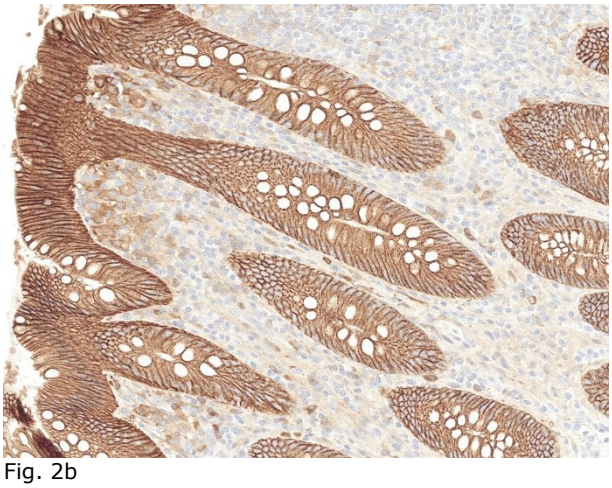


Fig. 2b
Insufficient ECAD staining reaction of the appendix using same protocol as in Fig. 1b.
Excessive background staining is seen and stromal cells (e.g. lymphocytes and plasma cells) in lamina propria mucosa display a weak to strong false positive membranous staining reaction.

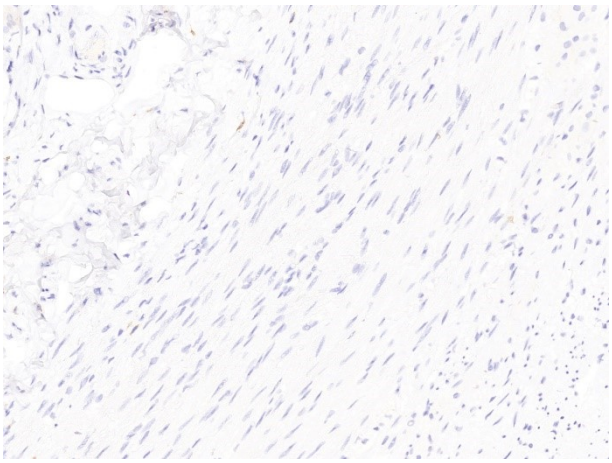


Fig. 3a
Optimal ECAD staining reaction of lamina muscularis in appendix using same protocol as in Fig. 1a. No staining reaction is observed.

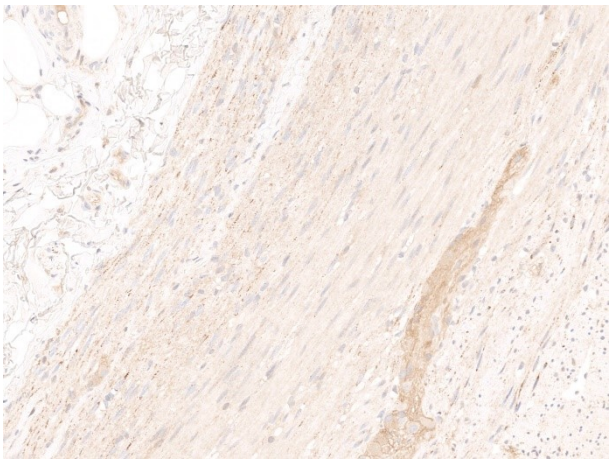


Fig. 3b
Insufficient ECAD staining reaction as the smooth muscle cells and nerves of lamina muscularis mucosae show an aberrant cytoplasmic staining reaction. Same protocol as in Fig. 1b.

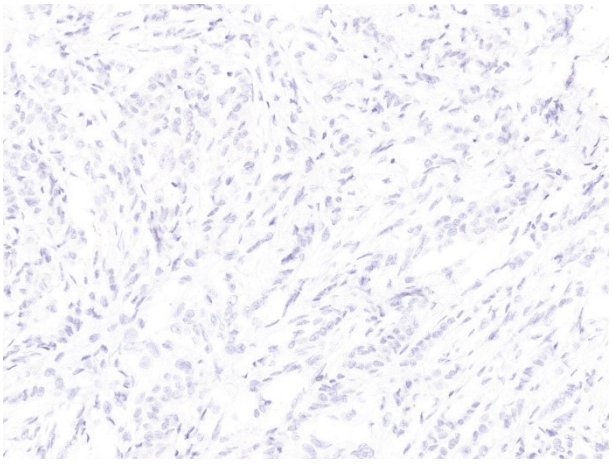


Fig. 4a
Optimal ECAD staining reaction of the lobular breast carcinoma, tissue core no. 5, using same protocol as in Figs. 1a – 3a. Virtually all the neoplastic cells are negative.

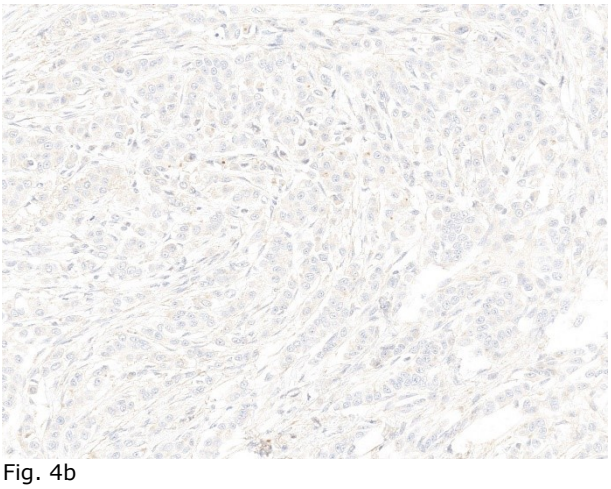


Fig. 4b
ECAD staining reaction of the lobular breast carcinoma, tissue core no. 5, using same protocol as in Figs. 1b – 3b. Although the neoplastic cells display similar reaction pattern as in Fig. 3a, a faint cytoplasmic staining reaction is observed.

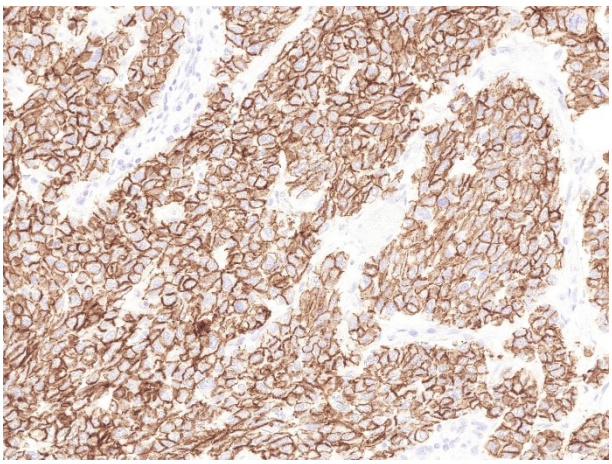


Fig. 5a
Optimal ECAD staining reaction of the ductal breast carcinoma using same protocol as in Fig. 1a. Virtually all neoplastic cells show a strong and distinct membranous staining reaction.

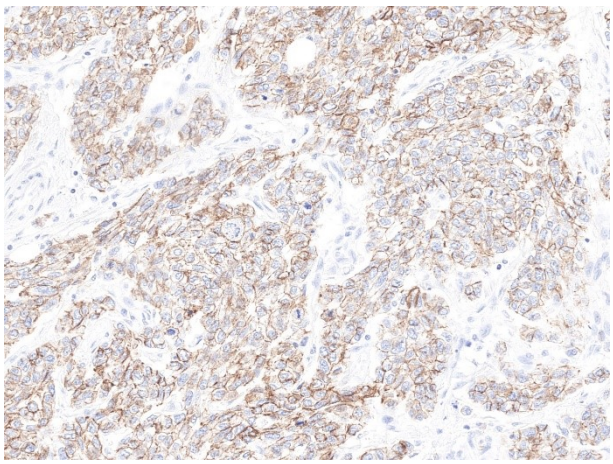


Fig. 5b
Insufficient staining reaction of ECAD in the ductal breast carcinoma using the RTU format (790-4497, Ventana/Roche) based on mAb 36 on a Benchmark platform, inefficient HIER (16 min.) performed in CC1 and OptiView used as detection system. The neoplastic cells display too weak membranous staining reaction. Compare with optimal result in Fig. 5a – same area.

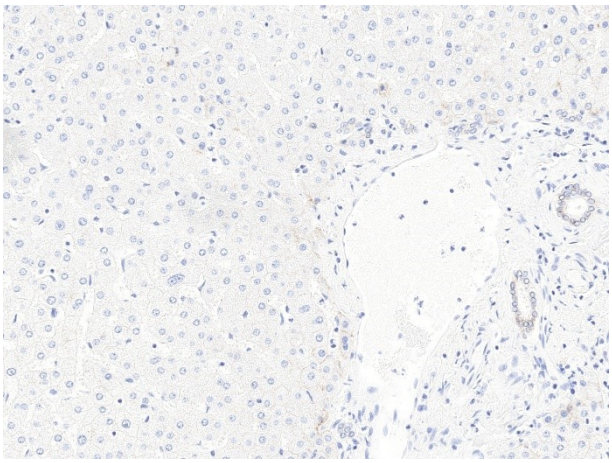


Fig. 6a
Insufficient ECAD staining reaction of liver using same protocol as in Fig. 5b. Hepatocytes are false negative. Compare to Fig. 1a for optimal result.

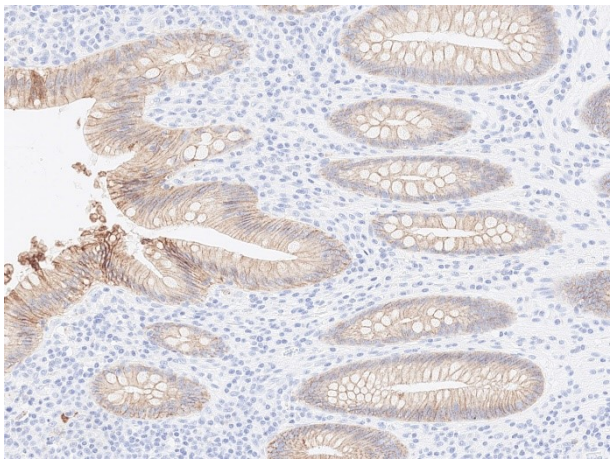


Fig. 6b
Insufficient ECAD staining of the appendix using same protocol as in Fig. 5b and 6a. A too weak staining reaction in epithelial cells is seen – compare with Fig. 2a for optimal result.

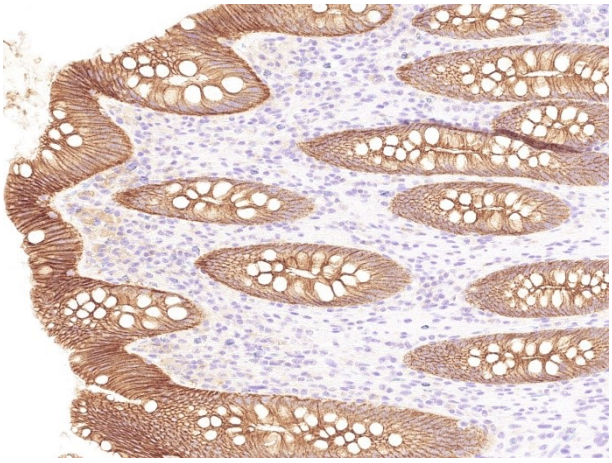


Fig. 7a
ECAD staining reaction of appendix using the RTU PA0387 on a Bond platform (Leica Biosystems) based on mAb clone 36B5, applying the recommended protocol settings HIER in BERS2 and Bond Refine as detection system.
The columnar epithelial cells display a moderate membranous staining reaction, and a weak background staining is seen in in lamina propria mucosa. Compare with Fig. 2a for optimal result.

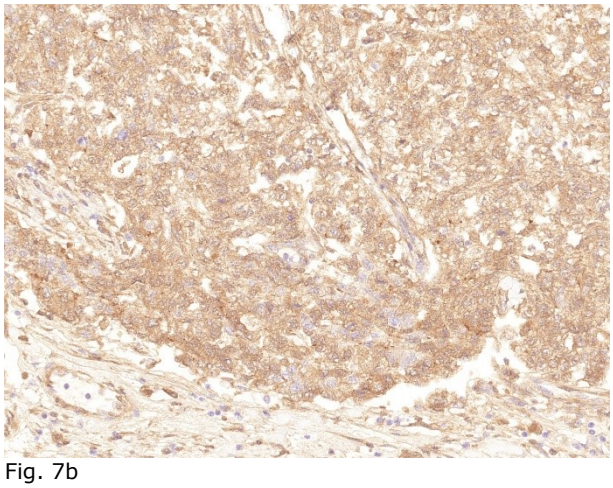


Fig. 7b
Insufficient ECAD staining reaction of the ductal carcinoma using same protocol as in Fig. 7a. An extended cytoplasmic staining reaction is observed, challenging the identification of true membrane reaction of the neoplastic cells. Compare with Fig. 5a for optimal results.

HLK/LE/SN 24.06.2025