Assessment Run C19 2026

Claudin 18.2 (CLDN18.2)

Purpose

This assessment of CLDN18.2 in the NordiQC Companion module focused on the evaluation of the analytical accuracy of the IHC assays performed by the NordiQC participants to identify patients with gastric and gastroesophageal junction (G/GEJ) cancer to be treated with VYLOY™. The technical accuracy was evaluated in six carcinomas with the dynamic and critical relevant expression levels of CLDN18.2 characterized by tumour cell scoring (TCS). The assessment mark obtained in NordiQC is indicative of the performance of the IHC tests but due to the limited number and composition of samples, internal validation/verification and extended quality control, e.g. regularly measuring the CLDN18.2 results, is needed.

Material

Table 1. **Content of the TMA used for the NordiQC CLDN18.2 C19 assessment**

Tissue controls	**CLDN18.2 IHC reaction pattern**
1. Gastric mucosa	See control section
2. Lung	See control section
Carcinomas	**TCS score***
3. Gastric carcinoma	<75%
4. Gastric carcinoma	≥75% (moderate to strong)
5. Gastric carcinoma	<75%
6. Gastric carcinoma	≥75% (moderate to strong)
7. Gastric carcinoma	<75%
8. Gastric carcinoma	≥75% (moderate to strong)

*Tumour Cell Scoring (TCS) determined by CLDN18 (43-14A) RxDx Assay (741-6067, Ventana/Roche) performed in NordiQC reference lab.

All tissues were fixed in 10% neutral buffered formalin.

KEY POINTS FOR CLDN18.2 IMMUNOASSAYS

- The Ventana/Roche RTU CLDN18 assays, based on clone 43-14A, provided a 100% pass rate overall.
- Laboratory developed assays based on concentrated Abs gave an inferior pass rate of 60%.
- Insufficient results were mainly caused by too weak or false negative staining reaction in gastric carcinomas expected to be TCS ≥75%

The participating laboratories were asked to perform their CLDN18.2 IHC assay for treatment decision with VYLOY™, evaluate the CLDN18.2 expression level using TCS as read-out method and submit the stained slides and scores to NordiQC. This allowed both an assessment of the technical performance (analytical accuracy) of the CLDN18.2 IHC assays but also information on the reproducibility and concordance of the CLDN18.2 expression read-out results among the laboratories.

CLDN18.2 IHC, Technical assessment

In order to account for heterogeneity of CLDN18.2 expression in the individual tumour cores included in the tissue micro array (TMA) blocks, reference slides were made throughout the blocks. Every twenty-fifth slide was stained for CLDN18.2 using the CE IVD / FDA approved CLDN18 (43-14A) RxDx Assay (741-6067, Ventana/Roche). During the assessment, TCS categories for each tissue core on the submitted slides were compared to the level in the nearest reference slide of CLDN18.2 (43-14A).

Criteria for assessing an IHC assay as <u>Optimal</u> include:

The result is considered perfect or close to perfect in all of the included tissues.
<u>TCS score is concordant to the NordiQC reference data in all neoplasias.</u>

Criteria for assessing an IHC assay as <u>Good</u> include:

The result is considered acceptable in all of the included tissues.
The CLDN18.2 expression in one or more tissues varies significantly from the expected scores, but still in the right category.
<u>TCS score is concordant to the NordiQC reference data in all neoplasias.</u>

Criteria for assessing an IHC assay as <u>Borderline</u> include:
The result is considered insufficient, e.g., because of generally too weak staining, a false negative staining or a false positive staining reaction of one of the included tissues.
<u>TCS score is **not** found concordant to the NordiQC reference data in all neoplasias.</u>

Criteria for assessing an IHC assay as <u>Poor</u> include:
The result is considered very insufficient e.g., because of a false negative or a false positive staining reaction in more than one of the included tissues.
<u>TCS score is **not** found concordant to the NordiQC reference data in all neoplasias.</u>

An IHC result could also be assessed as **Borderline/Poor**, if the signal-to-noise ratio was low, e.g., because of moderate cytoplasmic reaction, excessive/less-selective counterstaining or impaired morphology, to the extent where interpretation was compromised.

CLDN18.2 IHC, Read-out
All participating laboratories were asked to submit a scoring sheet with their read-out of the tumour cell scoring (TCS) in the six carcinomas using a ≥75% cut-off. Results were compared to NordiQC data from the reference laboratory to analyse scoring consensus.

Tumour Cell Scoring	What to Include
Cells Included	Greater than 50 Viable Tumour Cells Only
Staining Intensity	Moderate to Strong
Pattern of Staining	Apical, Circumferential (partial and complete), Basolateral/Lateral, Microluminal
Denominator	Total Number of Viable Tumour Cells
Scoring Algorithm for Gastric Adenocarcinoma Including the Gastroesophageal Junction	
Positive	≥75% viable tumour cells demonstrating moderate to strong membrane staining
Negative	<75% viable tumour cells demonstrating moderate to strong membrane staining

VENTANA CLDN18 (43-14A) RxDx Assay Interpretation Guide for Gastric Adenocarcinoma including GEJ, 1016391EN Rev A

Participation

Number of laboratories registered for CLDN18.2 IHC C19	129
Number of laboratories returning CLDN18.2 IHC slides	117 (91%)
Number of laboratories returning CLDN18.2 scoring sheet	106

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

Results: 117 laboratories participated in this assessment. 96% (112 of 117) achieved a sufficient mark. Assessment marks for IHC CLDN18.2 assays and CLDN18.2 antibodies are summarized in Table 2a-2d (see page 3-4).

Controls
Gastric mucosa was used as positive and negative tissue controls in concordance with the official scoring guidelines from Ventana/Roche. In gastric mucosa, virtually all normal epithelial cells should show a strong, membranous staining reaction. No staining should be seen in e.g. lymphocytes, smooth muscle cells and nerves. If the primary Ab also reacts with CLDN18.1 (as observed for the mAb clone 43-14A being used in the approved Ventana/Roche CDx assay for CLDN18.2), pneumocytes in normal lung show a weak membranous staining reaction. This reduced and low level CLDN18.1 expression in pneumocytes might be more valuable than intestinal metaplasia as a critical control for the evaluation of IHC reproducibility. However, more testing is needed to document the potential of lung/pneumocytes to serve as reliable control for CLDN18.2 IHC assays.

Conclusion
The CLDN18 (43-14A) RxDx Assays **741-6067** and **740-7037** and the RTU IHC assays **790-7027** and **744-7162** all from Ventana/Roche were the most successful assays for the evaluation of CLDN18.2 status in gastric carcinomas with an overall pass rate of 100%. Of the remaining CLDN18 assays, more clones could obtain optimal results, however, with only few observations and limited tissue cohort, the data must be carefully interpreted.

Table 2a. **Overall results for CLDN18.2, run C19**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
CE-IVD / FDA approved CLDN18.2 assays*	52	51	1	-	-	100%	98%
Antibodies for laboratory developed CLDN18.2 assays, based on concentrated antibodies	10	4	2	4	-	60%	40%
Ready-To-Use antibodies	55	46	8	-	1	98%	83%
Total	117	101	11	4	1		
Proportion		86%	10%	3%	1%	96%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
* Including all protocol settings - both performed as per recommended guidelines or modified settings.

Table 2b. **Assessment marks for CE-IVD / FDA approved CLDN18.2 assays, run C19**

CE-IVD / FDA approved CLDN18.2 assays	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone 43-14A, **741-6067[3]**	34	Ventana/Roche	34	-	-	-	100%	100%
mAb clone 43-14A, **741-6067[4]**	14	Ventana/Roche	13	1	-	-	100%	93%
mAb clone 43-14A, **740-7037[3]**	4	Ventana/Roche	4	-	-	-	-	-
Total	52		51	1	0	0		
Proportion			98%	2%	0%	0%	100%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
3) This product has a locked protocol on BenchMark XT/GX/Ultra and cannot be changed.
4) Product used on a non-intended platform.

Table 2c. **Assessment marks for concentrated antibodies for CLDN18.2, run C19**

Antibodies for laboratory developed CLDN18.2 assays, concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **43-14A**	5	Abcam	3	1	1	-	80%	60%
mAb clone **ABT-CLD18**	1	LS Bio	-	-	1	-	-	-
rmAb clone **QR120**	1	Quartett	-	-	1	-	-	-
rmAb clone **ZR451**	2	Zeta Corporation	1	1	-	-	-	-
Ab clone **BP6249**	1	Biolynx Biotechnology	-	-	1	-	-	-
Total	10		4	2	4	0		
Proportion			40%	20%	40%	0%	60%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).

Table 2d. **Assessment marks for Ready-To-Use antibodies for CLDN18.2, run C19**

Ready-To-Use antibodies[7]	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone 43-14A, **744-7162[3]**	5	Ventana/Roche	5	-	-	-	100%	100%
mAb clone 43-14A, **790-7027 (VRPS)[5]**	21	Ventana/Roche	21	-	-	-	100%	100%
mAb clone 43-14A, **790-7027 (LMPS)[6]**	19	Ventana/Roche	16	3	-	-	100%	84%
mAb clone **127E6G8 PA608**	1	Abcarta	1	-	-	-	-	-
mAb clone **MX153 MAB-1174**	1	Fuzhou Maixin	1	-	-	-	-	-
rmAb clone ZR451, **Z2807RP**	3	Zeta Corporation	1	2	-	-	-	-
rmAb clone DBRRM1.82, **RMPD116R**	2	Diagnostic BioSystems	-	2	-	-	-	-
rmAb clone **QR120 P-C049-30**	1	Quartett	-	-	-	1	-	-
rmAb clone **RM510 GT2441**	1	Gene Tech	-	1	-	-	-	-
Ab clone **C7X3 CCR-1272**	1	Celnovte	1	-	-	-	-	-
Total	55		46	8	0	1		
Proportion			83%	15%	0%	2%	98%	

1) Proportion of sufficient results (optimal or good) (≥5 assessed protocols).
2) Proportion of optimal results (≥5 assessed protocols).
3) This product has a locked protocol on BenchMark XT/GX/Ultra/Ultra Plus and cannot be changed.
5) Vendor recommended protocol settings – RTU product used in compliance to protocol settings, platform and package insert.
6) Laboratory modified protocol settings for a RTU product applied either on the vendor recommended platform(s) or other platforms.
7) Ready-To-Use antibodies without predictive claim.

Detailed Analysis

CE IVD / FDA approved assays

43-14A (741-6067, Ventana/Roche): In total, 34 of 34 (100%) protocols were assessed as optimal. This product has a locked protocol on BenchMark Ultra/XT/GX and cannot be changed. The protocol is based on Heat Induced Epitope Retrieval (HIER) in Cell Conditioning 1 (CC1) for 64 min., 16-32 min. incubation of primary Ab depending on the platform and OptiView as detection system. Using these protocol settings and applied on BenchMark XT/GX/Ultra, 34 of 34 (100%) laboratories produced a sufficient staining result, all optimal.
The product was used by 14 laboratories on a non-intended platform. This data is not included.

Ready-To-Use antibodies for laboratory developed (LD) assays

43-14A (790-7027, Ventana/Roche): In total, 27 of 27 (100%) protocols performed on the intended BenchMark XT/GX/Ultra provided an optimal result. Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 32-64 min.), 16 min. incubation of primary Ab and OptiView as detection system. Using these settings, 27 of 27 (100%) produced an optimal staining result.
The product was used by 13 laboratories on a non-intended platform. This data is not included.

Block construction and assessment reference standards
The tissue micro array (TMA) blocks constructed for this CLDN18.2 run consisted of six gastric carcinomas, one gastric mucosa and one normal lung. The six gastric carcinomas were selected to comprise three carcinomas with a TCS ≥75% and three with TCS score <75%.
Reference slides throughout the individual TMA blocks (interval at each twenty-fifth slide) were stained using the companion diagnostic assay CLDN18 (43-14A) RxDx Assay (741-6067, Ventana/Roche).
In total, four identical TMA blocks were constructed and used for this assessment.
During the assessment, TCS for each tissue core on the submitted slides were compared to the level in the nearest reference slides.

Comments – accuracy of CLDN18.2 IHC using TCS scoring to guide treatment with VYLOY™
In this NordiQC run for CLDN18.2 in the companion module, a pass rate of 96% was observed for the participants performing CLDN18.2 IHC assays to identify patients with gastric carcinomas eligible for treatment with VYLOY™ using the TCS method.

It was observed that insufficient results were most frequently characterized by a reduced proportion of cells demonstrated or a completely false negative staining reaction of neoplastic cells in one or more of the tissue cores and was seen in 60% (3 of 5) of the insufficient results. This was especially observed in tissue cores 4 and 8. In the remaining 40% (2 of 5), the insufficient staining result was caused by both aberrant nuclear staining and cytoplasmic staining, mainly seen in tissue core 5.

The Ventana/Roche CLDN18 (43-14A) RxDx Assays **741-6067** and **740-7037** (BenchMark Ultra/XT/GX) with predictive claim for VYLOY™ was used by 32% (38 of 117) of the participants on the intended platform and provided a pass rate of 100%, all optimal. The assay is locked in central protocol settings and based on HIER in CC1 for 64 min., incubation in primary Ab for 16-32 min., depending on platform, and use of OptiView as detection system for BenchMark XT/GX/Ultra.
13 laboratories used 741-6067 on the BenchMark Ultra plus (off label use), applying similar protocol settings to the recommendations. All with sufficient results.

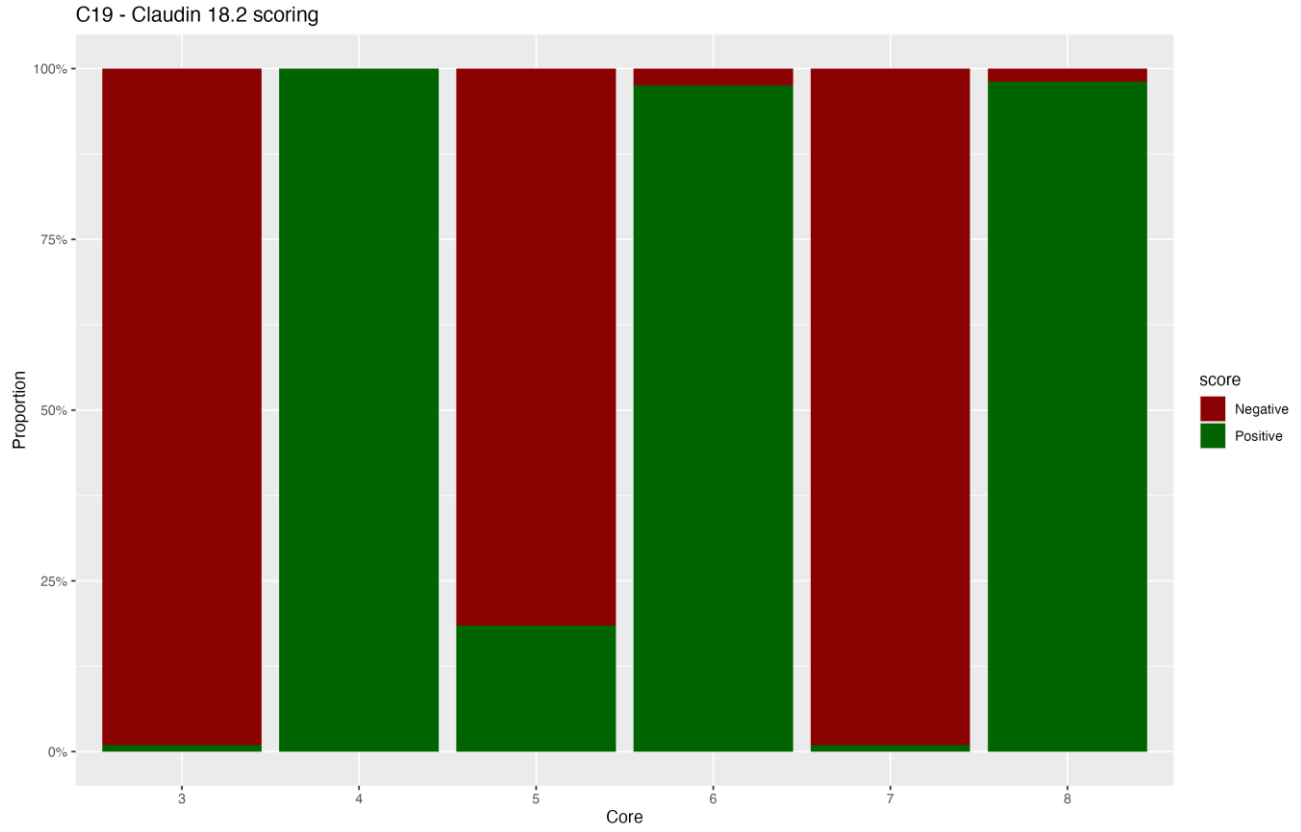
The Ventana/Roche CLDN18 43-14A assay **790-7027** (BenchMark Ultra/XT/GX) without predictive claim and available as an analytical or generic CLDN18.2 assay was used by 23% (27 of 117) of the participants on the intended platform. This assay is based on the same recommended protocol settings as the corresponding CDx products 741-6067/740-7037, but with ordinary options for the laboratories to modify the protocol settings in their optimization and validation process for the implementation of the test. Overall, the CLDN18.2 790-7027 format also gave a pass rate of 100% as the locked assays. A similar pass rate and proportion of optimal results was obtained, when using the vendor recommended protocol settings, compared to the CDx formats of the same clone as seen in Table 2b + 2d (see page 3-4).
13 laboratories used the 790-7027 on another platform than developed for, including 10 on BenchMark Ultra plus, all with a sufficient result.

Laboratory developed (LD) assays based on concentrated primary Abs gave an overall significantly inferior performance and reduced pass rate of 60% (6 of 10), 40% optimal (n=4). Within LD-assays, and no matter which Ab clone is used, meticulous calibration and validation of the assay is required. Internal NordiQC studies have e.g. shown that different Ab clones for CLDN18.2 give different staining patterns in normal tissues, which must be taken into account when evaluating the reaction pattern and to verify if the result is as expected in clinical tissues.

Five laboratories used the concentrated format of **mAb clone 43-14A (Abcam)**, 80% with a sufficient result. The submitted protocol settings were all based on HIER in an alkaline buffer and a 3- or 4-layer detection system. The titre ranged from 1:1,000-8,000, and with only few observations on three different platforms, it is not possible to identify an optimal and robust, recommendable protocol for the different commercially available IHC platforms.

CLDN18.2 scoring
Participants were asked to evaluate the TCS in each of the six gastric carcinomas (TCS with 75% cut-off) included in the assessment material. The overall read-out of the CLDN18.2 expression among the participants is shown in Graph 1.

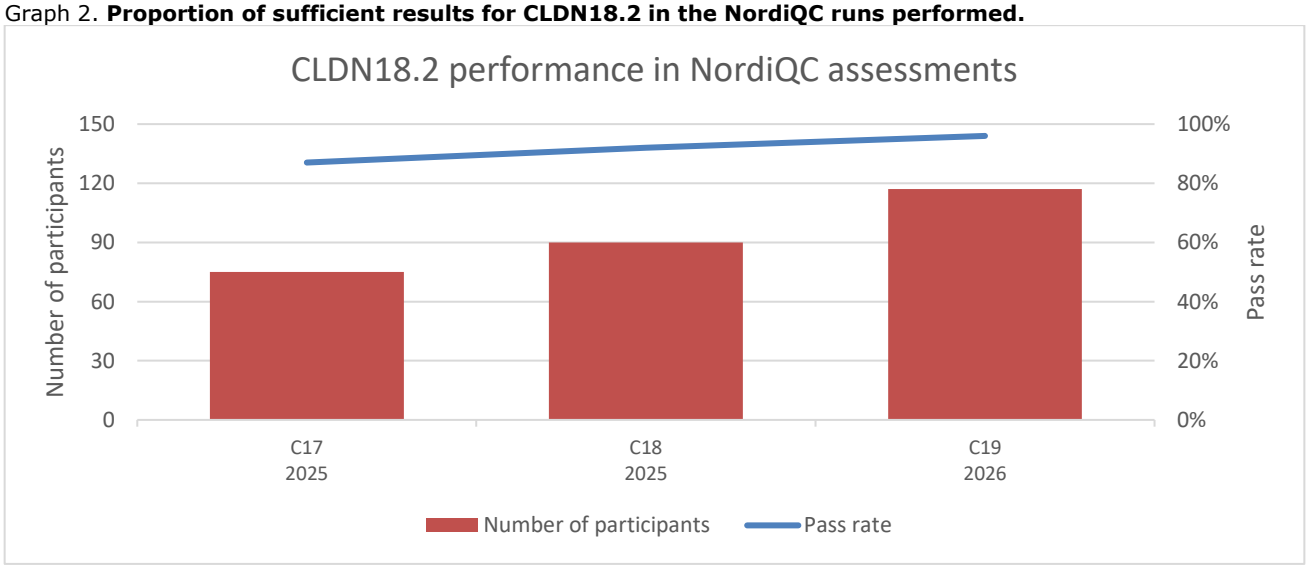
Graph 1. **NordiQC CLDN18.2 run C19: Read-out of TCS in six gastric carcinomas.**



As seen in Graph 1, consensus rates were generally high. Tissue cores 3 and 7 (TCS <75%), as well as 4, 6 and 8 (TCS ≥75%), were scored similarly by nearly all participants. The slightly lower consensus rate for tissue core 5 may have been caused by a tumour with 30-40% moderate to strong CLDN18.2 expression.

Performance history: This was the third NordiQC assessment of CLDN18.2 in NordiQC. Compared to C18, a slightly improved pass rate was obtained in C19 (see Graph 2).

Graph 2. **Proportion of sufficient results for CLDN18.2 in the NordiQC runs performed.**



Summary

This was the third NordiQC assessment of CLDN18.2 for TCS in gastric carcinomas in the NordiQC companion module. 117 laboratories participated and an increased pass rate of 96% was observed, despite an increased number of participants (see Graph 2).

The CLDN18 (43-14A) RxDx Assays 741-6067 and 740-7037 and the RTU IHC assays 790-7027 and 744-7162 all from Ventana/Roche were the most successful assays for the evaluation of CLDN18.2 status in gastric carcinomas with an overall pass rate of 100%. In total 23 laboratories applied the assay on the non-intended BenchMark Ultra plus, all with similar protocol settings to the vendor recommendations, and all with sufficient results.

Of the remaining CLDN18 assays, more clones could obtain optimal results, however, with only few observations and limited tissue cohort, the data must be carefully interpreted.

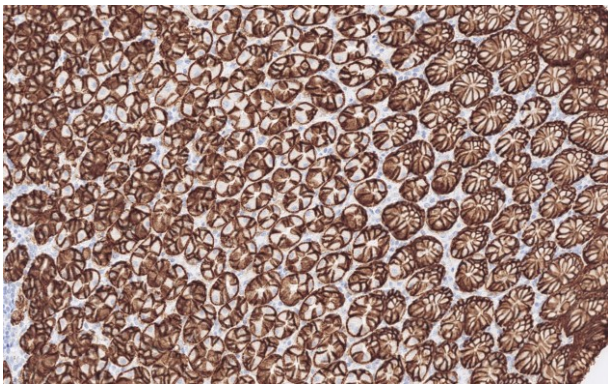


Fig. 1a
Optimal staining result of gastric mucosa using the CLDN18 RxDx IHC assay 741-6067 from Ventana/Roche, based on the mAb clone 43-14A following the recommended protocol settings. Same protocol used in Figs. 2a-5a.
Virtually all epithelial cells show a moderate to strong membranous staining reaction.

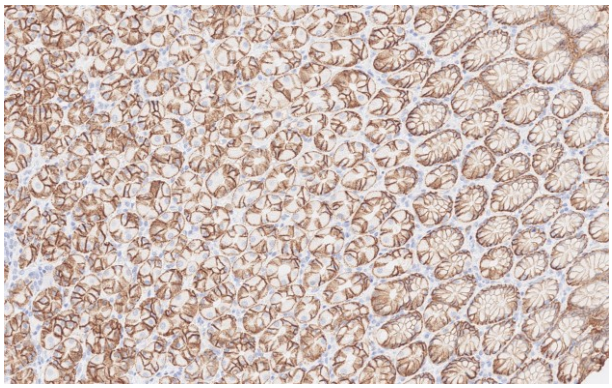


Fig. 1b
Staining result of gastric mucosa using the mAb clone ABT-CLD18 as an LD assay. The Ab was diluted 1:2000, HIER was performed in an alkaline buffer, and a 3-layer detection system was used. Same protocol used in Figs. 2b-5b.
The staining intensity of epithelial cells is significantly reduced compared to the optimal result in Fig. 1a.

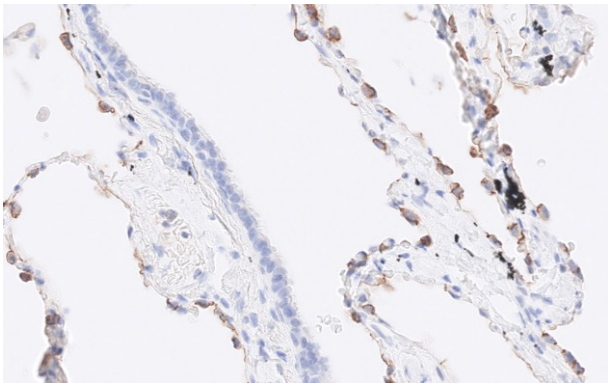


Fig. 2a
Optimal staining result of normal lung, using the same protocol as in Fig. 1a. Pneumocytes show a weak to moderate membranous staining reaction. Note this reaction is only seen for Abs also directed against CLDN18.1

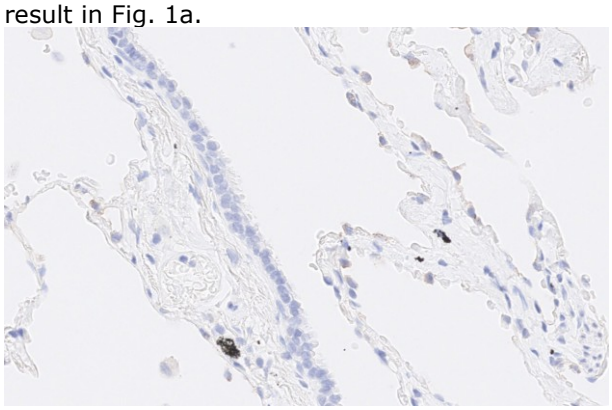


Fig. 2b
Staining result of normal lung, using the same protocol as in Fig. 1b. A significantly reduced proportion of pneumocytes show a faint staining reaction compared to the optimal result in Fig. 2a. Note this reaction is only seen for Abs also directed against CLDN18.1

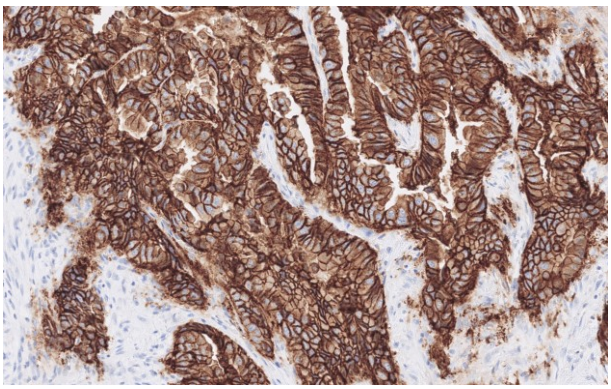


Fig. 3a
Optimal staining result of gastric carcinoma, tissue core 4, using the same protocol as in Figs. 1a – 2a.
A moderate to strong, membranous staining reaction is seen in ≥75% of the neoplastic cells and thus tumour being eligible for treatment with VYLOY™.

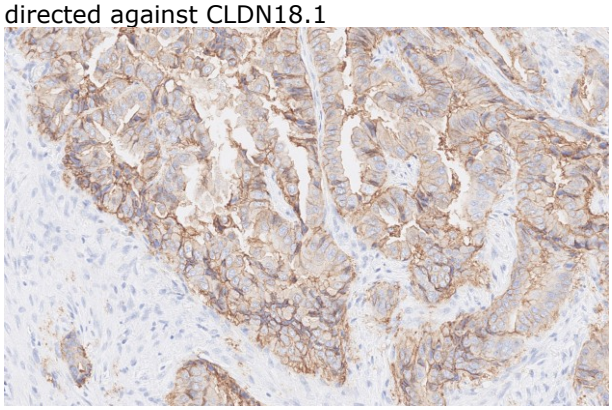


Fig. 3b
Insufficient staining result of gastric carcinoma, tissue core 4, using same protocol as in Figs. 1b – 2b. The neoplastic cells show a too weak staining reaction, changing the TCS from positive to negative and tumour not being eligible for treatment with VYLOY™.
Compare with Fig. 3a – same area.

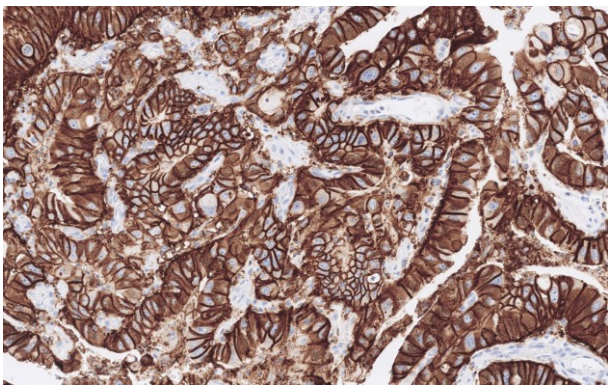


Fig. 4a
Optimal staining result of the gastric carcinoma, tissue core 8, using same protocol as in Figs. 1a-3a. A moderate to strong, membranous staining reaction is seen in ≥75% of the neoplastic cells and thus tumour being eligible for treatment with VYLOY™.

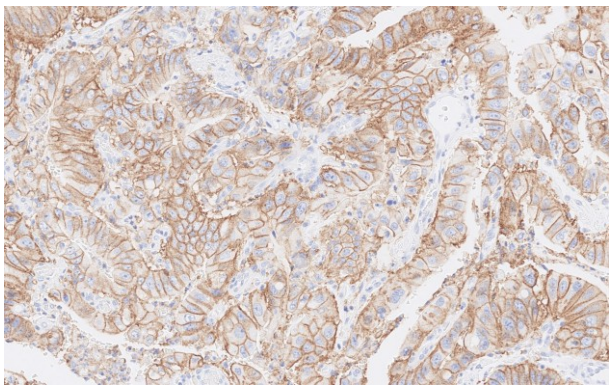


Fig. 4b
Staining result of the gastric carcinoma, tissue core 8, using same protocol as in Figs. 1b-3b. The neoplastic cells show a weaker membranous staining reaction than expected. Overall, the result in the tumour was still scored as TCS high, but the sensitivity is reduced.
Compare with Fig. 4a – same area.

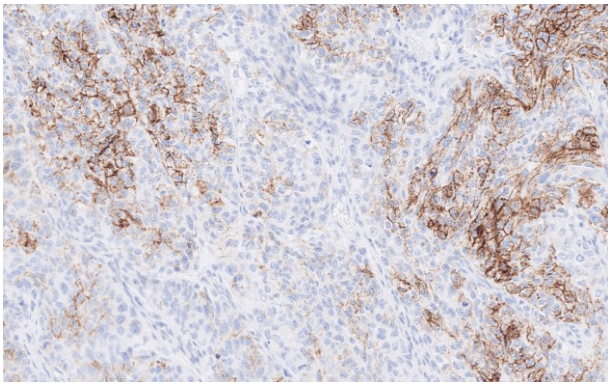


Fig. 5a
Optimal staining result of the gastric carcinoma, tissue core 5, using same protocol as in Figs. 1a – 4a. A weak to moderate membranous staining reaction is seen in about 40% of the neoplastic cells and tumour being TCS negative.

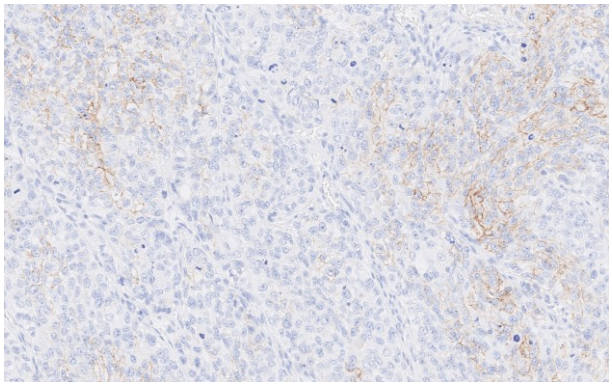


Fig. 5b
Staining result of the gastric carcinoma, tissue core 5, using same protocol as in Figs. 1b – 4b. A significantly reduced intensity and proportion of positive cells are seen compared with optimal result in Fig. 5a – same area.

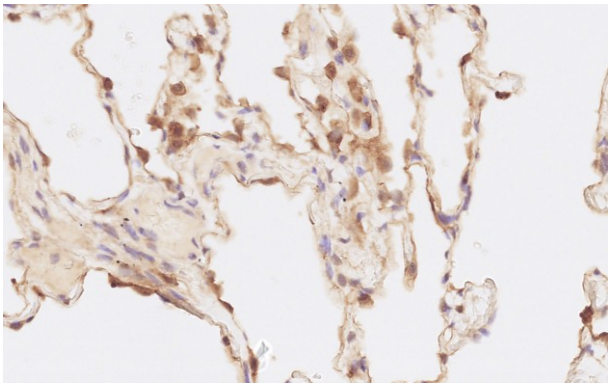


Fig. 6a
Insufficient staining result of normal lung using rmAb clone QR120. HIER was performed in an alkaline buffer, and a 2-layer detection system was used. Both a cytoplasmic and nuclear staining reaction were seen. Compare to optimal result in Fig. 2a.

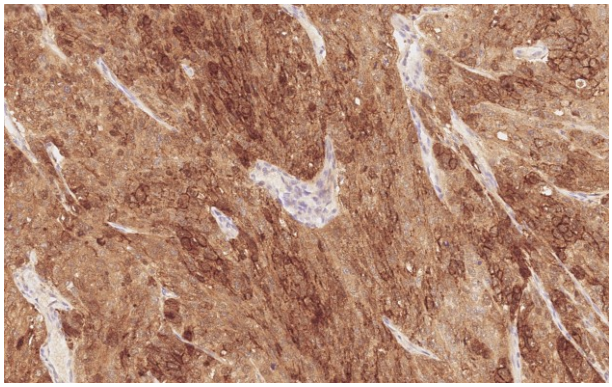


Fig. 6b
Insufficient staining result of the gastric carcinoma core 5, using same protocol as in Fig. 6a. A moderate cytoplasmic staining reaction is seen complicating the interpretation of the membranous staining reaction. Compare with optimal result in Fig. 5a.

HLK/TJU/KBA/RR 03.07.2026