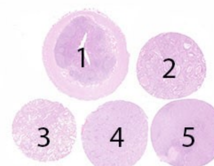
NordiQC

Assessment Run 73 2025
Lung Anaplastic Lymphoma Kinase (ALK (lung))

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests among NordiQC participants for EML4-ALK translocations in lung adenocarcinomas. The EML4-ALK translocation occurs through a paracentric inversion between EML4 and ALK genes located in the short arm of chromosome 2 and induces an EML4-ALK fusion protein being expressed in 2–4% of lung adenocarcinomas and is a target for ALK tyrosine inhibitors as crizotinib, ceritinib, and alectinib.

Material
The slide to be stained for ALK (lung) comprised:

1. Appendix, 2. Lung adenocarcinoma without EML4-ALK translocation*, 3. Lung adenocarcinoma with EML4-ALK translocation*, 4. Merkel cell carcinoma, 5. Anaplastic large cell lymphoma (ALCL) with ALK translocation*.
*confirmed by Next Generation Sequencing (NGS)



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing ALK (lung) staining as optimal included:

- A distinct, moderate to strong nuclear and cytoplasmic staining reaction of virtually all neoplastic cells in the anaplastic large cell lymphoma (ALCL).
- An at least weak to moderate granular cytoplasmic staining reaction of virtually all neoplastic cells in the lung adenocarcinoma with EML-ALK translocation.
- An at least weak to moderate granular cytoplasmic staining reaction with membrane accentuation of virtually all neoplastic cells in the Merkel cell carcinoma.
- An at least weak to moderate granular cytoplasmic staining reaction of ganglion cells and dispersed axons in the appendix.
- No staining of neoplastic cells in the lung adenocarcinoma without ALK rearrangement.
- No staining of epithelial cells in the appendix and tonsil.

KEY POINTS FOR ALK (lung) IMMUNOASSAYS
- The mAb clone **OTI1A4** is recommendable both as a concentrated Ab and a RTU.
- The mAb clone **ALK-1** should not be used for lung adenocarcinomas.
- Appendix is a recommendable, external positive tissue control

Participation

Number of laboratories registered for ALK (lung), run 73	330
Number of laboratories returning slides	307 (93%)

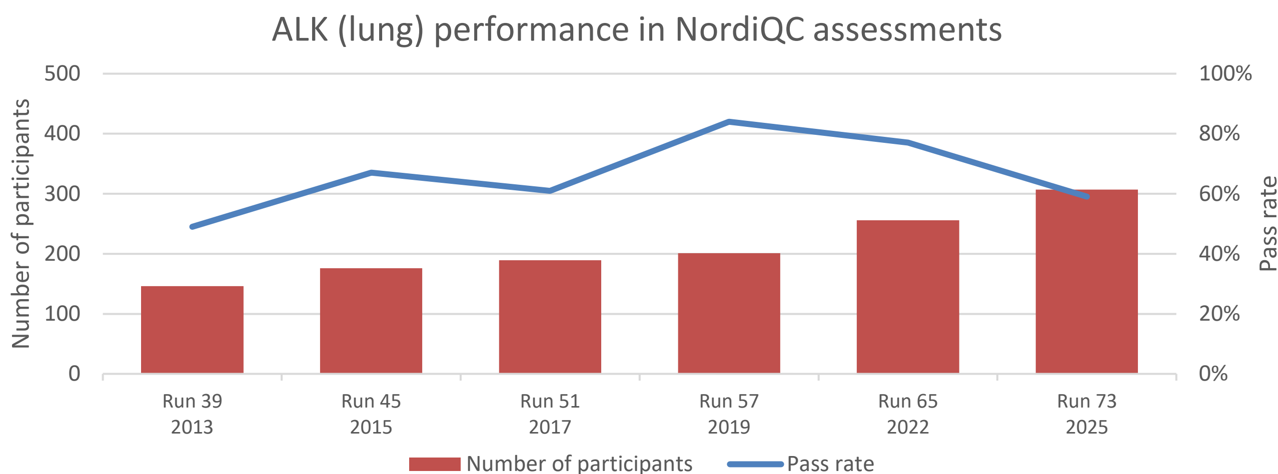
Results
At the date of assessment, 93% of the participants had returned the circulated NordiQC slides. All slides returned after the assessment were assessed and laboratories received advice if the result was insufficient, but the data were not included in this report.

307 laboratories participated in this assessment. 180 (59%) achieved a sufficient mark (optimal or good), see Table 1a (see page 3). Tables 1b and 1c summarizes the antibodies (Abs) used and assessment marks (see page 3-4).

The most frequent causes of insufficient staining reactions were:
- Unexpected and aberrant cytoplasmic staining reaction on the BenchMark platform.
- Less successful primary antibodies (mAb clone ALK1)

Performance history
This was the sixth NordiQC assessment of ALK (lung). A significant decrease of the pass rate was seen compared to run 57 (2019) and run 65 (2022) (see Graph 1).

Graph 1. **Proportion of sufficient results for ALK (lung) in the six NordiQC runs performed**



Conclusion
The mAb clone **OTI1A4** is highly recommendable for demonstration of EML4-ALK translocation in lung adenocarcinoma. Heat Induced Epitope Retrieval (HIER) at high pH, use of a sensitive 3-step polymer/multimer based detection system and appropriate calibration of the titer of the primary antibody were crucial for an optimal performance. Optimal staining results were also seen with the mAb clone **5A4** and rmAb clone **D5F3**, but especially for mAb clone 5A4, the analytical sensitivity was lower compared to mAb clone OTI1A4.
The Dako/Agilent Ready-To-Use (RTU) system based on the mAb clone OTI1A4 was the most successful assay with a pass rate of 100%, 84% optimal when applied within vendor recommended protocol settings. The Ventana/Roche RTU system based on the rmAb clone D5F3 was the most used assay and using the recommended protocol settings providing an overall pass rate of 57%. The low pass rate was primarily caused by an unexpected, aberrant cytoplasmic staining reaction in epithelial cells of appendix compromising the analytical specificity and final readout.

Controls
In this assessment and in concordance with previous assessments, appendix was found to be a valuable and recommendable external positive tissue control, especially useful to evaluate the level of the analytical sensitivity of the assay: In virtually all optimal protocols for ALK (lung), a weak to strong granular cytoplasmic staining reaction was seen in the ganglion cells and a weak to moderate reaction in the axons. If these cells/structures were negative, a too weak or even completely false negative staining reaction was seen in the lung adenocarcinoma with EML4-ALK translocation and the Merkel cell carcinoma.
In general, the mAb clone OTI1A4 and rmAb clone D5F3 gave a stronger and more extensive staining reaction of ganglion cells compared to mAb clone 5A4. This could reflect a higher analytical sensitivity of these two clones.
In this assessment and in concordance with the previous assessments, the Merkel cell carcinoma proved to be challenging. Merkel cell carcinomas do not harbour ALK translocations/inversions, but more than 90% show aberrant/overexpression of ALK protein[1,2]. The amount of ALK protein is generally much lower than in ALCL, most often on par with low level ALK expressing lung adenocarcinoma with EML4-ALK translocation. This makes Merkel cell carcinomas an important addition to the positive tissue controls needed for ALK (lung) assays, at least for the initial calibration/validation process.

[1] Filtenborg-Barnkob BE, Bzorek M. Expression of anaplastic lymphoma kinase in Merkel cell carcinomas. Hum Pathol. 2013 Jul 31;44(8):1656–64.
[2] Veija T, Koljonen V, Bohling T, Kero M, Knuutila S, Sarhadi VK. Aberrant expression of ALK and EZH2 in Merkel cell carcinoma. BMC Cancer. 2017 Mar 31;17(1):236.

Table 1a. **Overall results for ALK (lung), run 73**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	56	4	24	22	6	50%	7%
Ready-To-Use antibodies	251	59	93	70	29	60%	23%
Total	307	63	117	92	35		
Proportion		21%	38%	30%	11%	59%	

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

Table 1b. **Concentrated antibodies and assessment marks for ALK (lung), run 73**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **5A4**	1	Abcam	0	0	1	0	-	-
	1	Biocare Medical	0	0	1	0	-	-
	1	Diagnostic BioSystems	0	0	1	0	-	-
	17	Leica Biosystems	1	8	8	0	39%	4%
	1	Master Diagnostica	0	0	0	1	-	-
	1	Monosan	0	0	1	0	-	-
	1	Zytomed Systems	0	0	1	0	-	-
mAb clone **OTI1A4***	7	Origene	3	4	0	0	100%	43%
mAb clone **IHC509**	1	GenomeMe	0	0	1	0	-	-
rmAb clone **D5F3**	18	Cell Signaling	0	10	8	0	56%	0%
rmAb clone **ALK1**	5	Dako/Agilent	0	1	0	4	20%	0%
rmAb clone **QR017**	1	Quartett	0	1	0	0	-	-
rmAb clone **ZR305**	1	Zeta Corporation	0	0	0	1	-	-
Total	56		4	24	22	6		
Proportion			7%	43%	39%	11%	50%	

1) Proportion of sufficient stains (optimal or good) (≥5 assessed protocols).
2) Proportion of Optimal Results (≥5 assessed protocols).
*) OTI1A4 is called 1A4 by some vendors

Table 1c. **Ready-To-Use antibodies and assessment marks for ALK (lung), run 73**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **5A4** **PA0831 (VRPS)[3]**	9	Leica Biosystems	4	4	1	0	89%	44%
mAb clone **5A4** **PA0831 (LMPS)[4]**	15	Leica Biosystems	2	10	1	2	80%	13%
mAb clone **5A4** **API3041**	1	BioCare	0	0	1	0	-	-
mAb clone **5A4** **CAM-0170**	1	Celnovte	0	1	0	0	-	-
mAb clone **5A4** **MAD-001720QD**	2	Master Diagnostica	0	0	1	1	-	-
mAb clone **ALK1** **GA641 (VRPS)[3]**	4	Dako/Agilent	0	0	0	4	-	-
mAb clone **ALK1** **GA641 (LMPS)[4]**	6	Dako/Agilent	0	0	1	5	0%	0%
mAb clone **ALK1** **IR641**	3	Dako/Agilent	0	0	0	3	-	-
mAb clone **ALK1** **790/800-2918 (VRPS)[3]**	1	Ventana/Roche	0	0	0	1	-	-
mAb clone **ALK1** **790/800-2918 (LMPS)[4]**	10	Ventana/Roche	0	1	1	8	10%	0%
mAb clone **ALK1** **204M-17/18**	1	Cell Marque	0	0	0	1	-	-
mAb clone **137E9E8** **PA132**	1	Abcarta	1	0	0	0	-	-
mAb clone **OTI1A4*** **8344-C010**	2	Sakura Finetek	0	2	0	0	-	-
mAb clone **OTI1A4*** **GA785 (VRPS)[3]**	32	Dako/Agilent	27	5	0	0	100%	84%
mAb clone **OTI1A4*** **GA785 (LMPS)[4]**	11	Dako/Agilent	6	4	1	0	91%	55%
mAb clone **4A4** **PDM566**	2	Diagnostic BioSystems	0	1	1	0	-	-
mAb clone **MX064** **MAB-1156**	1	Maixin	0	0	1	0	-	-
rmAb clone **D5F3** **790-4794/970-4796/ 741-7157 (VRPS)[3]**	110	Ventana/Roche	14	49	46	1	57%	13%
rmAb clone **D5F3** **790-4794/970-4796/ 741-7157 (LMPS)[4]**	35	Ventana/Roche	5	13	15	2	51%	14%
rmAb clone **SP8** **AN770-5/10M**	1	BioGenex	0	0	0	1	-	-
rmAb clone **BY080** **BFM-0175**	1	Bioin Biotechnology	0	1	0	0	-	-
Ab clone **DY49198** **4911702**	1	Dakewe	0	1	0	0	-	-
Ab clone **1H7** **GT231102**	1	Gene Tech	0	1	0	0	-	-
Total	251		59	93	70	29		
Proportion			23%	37%	28%	12%	60%	

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).
*) OTI1A4 is called 1A4 by some vendors

Detailed analysis of ALK (lung), Run 73
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **OTI1A4**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) High pH (Dako/Agilent) (3/5)* as retrieval buffer. The mAb was diluted in the range of 1:500-1:1,000 and a 3-step detection system was applied. Using these protocol settings, 4 of 4 laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **5A4**: One protocol obtained an optimal result, based on HIER using TRS High pH (Dako/Agilent) (1/1) as retrieval buffer. The mAb was diluted 1:25 and a 3-step detection system was applied. The protocol was applied on the Dako Autostainer platform.

Table 3. **Proportion of optimal results for ALK (lung) for the most commonly used antibodies as concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako/Agilent Autostainer[1]**		**Dako/Agilent Omnis**		**Ventana/Roche BenchMark[2]**		**Leica Biosystems Bond**	
	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 **OTI1A4**	1/1	-	2/3	-	0/1	-	-	-
mAb clone **5A4**	1/1	-	-	-	0/2	-	-	-

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** (number of optimal results/number of laboratories using this buffer)
1) Autostainer Classic.
2) BenchMark Ultra.

Ready-To-Use antibodies and corresponding systems
mAb clone **5A4,** product no. **PA0831**, Leica Biosystems, Bond III / Prime:
Protocols with optimal results were based on HIER using Bond Epitope Retrieval 2 (BERS2) (efficient heating time 20-30 min. at 99-100°C), 15-30 min. incubation of the primary Ab and Bond Polymer Refine (DS9800) as detection system. Using these protocol settings, 16 of 18 (89%) produced a sufficient staining result (optimal or good).

mAb clone **OTI1A4,** product no. **GA785**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH (efficient heating time 30 min. at 97-99°C), 20-30 min. incubation of the primary Ab and EnVision Flex+ (GV800/GV823+GV821) as detection system. Using these protocol settings, 40 of 41 (98%) produced a sufficient staining result.

rmAb clone **D5F3** product no. **790-4794/970-4796/741-7157**, Ventana/Roche, BenchMark GX, XT, Ultra and Ultra Plus:
Protocols with optimal results were typically based on HIER using Cell Conditioning 1 (CC1) (efficient heating time 92 min.), 16 min. incubation of the primary Ab. and OptiView (760-700) + amplification kit (760-099) as detection system. Using these protocol settings, 75 of 131 (57%) laboratories produced a sufficient staining result.

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems. 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 specific IHC stainer device are included.

Table 4. **Proportion of sufficient and optimal results for ALK (lung) for the most commonly used RTU IHC systems**

RTU-systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
VMS Ultra/XT rmAb D5F3 **790-4794/970-4796/741-7157**	57% (63/110)	13% (14/110)	45% (14/31)	16% (5/31)
Dako Omnis mAb OTI1A4 **GA785**	100% (32/32)	84% (27/32)	89% (8/9)	67% (6/9)
Leica BOND mAb 5A4 **PA0831**	89% (8/9)	44% (4/9)	83% (10/12)	17% (2/12)

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer were included.

Comments

In this assessment, the prevalent feature of an insufficient result was an aberrant cytoplasmic staining reaction in epithelial cells of appendix and was seen in 51% (65 of 127) of the insufficient results, complicating the interpretation. This staining pattern was only observed on Ventana BenchMark platforms, thus with various primary Abs. In 45% (57 of 127) of insufficient results a too weak or false negative staining reaction of cells expected to be demonstrated was observed. The remaining 4% were characterized by a poor signal-to-noise ratio or excessive background staining reaction compromising the interpretation. Virtually all the participating laboratories were able to demonstrate ALK in the neoplastic cells of the ALCL, whereas the Merkel cell carcinoma and the lung adenocarcinoma with EML-4 ALK translocation were more challenging and required an optimally calibrated IHC system.

18% (56 of 307) of the laboratories used Abs as concentrated formats within laboratory developed (LD) assays for ALK. The mAb clones 5A4, OTI1A4 and the rmAb clone D5F3 were the most widely used antibodies (see Table 1b). In concordance with previous assessments, mAb clone **OTI1A4** was especially successful with a general pass rate of 100% (7 of 7) with 43% optimal. Optimal staining results were obtained on Dako/Agilent Autostainer and Omnis (see Table 3).
The rmAb clone **D5F3** obtained a pass rate of 56% (10 of 18), thus no optimal results. Sufficient staining results were obtained on all fully automated platforms from the main vendors, using HIER in an alkaline buffer and a use of a sensitive 3-step detection system.
The pass rate of mAb clone **5A4** has decreased through the latest assessments. In 2019 (Run 57) and 2022 (Run 65) pass rates of 81% and 49% were obtained, respectively. In this run, a pass rate of 39% (9 of 23) was seen, 4% optimal (n=1). No obvious reason for the reduced pass rate has been identified. The mAb clone 5A4 gave sufficient staining results on 3 of the 4 main IHC systems - no laboratories used the clone on the Dako Omnis system. For the mAb clone 5A4, efficient HIER in an alkaline buffer, careful calibration of the titer of the primary Ab and especially use of a sensitive 3-step polymer/multimer based detection system were the main prerequisites for a sufficient and optimal staining result similar to the observations for the Ab clones D5F3 and OTI1A4.

82% (251 of 307) of the laboratories used Abs as Ready-To-Use (RTU) formats. The Ventana RTU system based on the rmAb clone D5F3 (prod. no. **790-4794/790-4796/741-7157**) was the most used assay for ALK giving an overall pass rate of 55% (77 of 141 laboratories) with 14% optimal, which is a significant decrease from the last Run 65 with a pass rate of 94%, 81% optimal. In this run 73, an aberrant cytoplasmic staining reaction in epithelial cells of appendix was seen in 79% (111 of 141) of the participants in various degree, complicating the interpretation and questioning the analytical specificity of the IHC assay. No obvious reason for this aberrant staining pattern has been identified. 46 laboratories, using the Ventana RTU system, used an "in-house" appendix as an on-slide control. In 89% (41 of 46) of the on-slide controls same aberrant cytoplasmic staining reaction was observed in the epithelial cells, indicating it is not an isolated incident related to the NordiQC tissue material. At this point no conclusions on the cause for the aberrant cytoplasmic staining reaction in cells not expected to be positive has been generated. Most likely the reaction could be related to a cross-reaction from either the primary Ab/RTU format or the OptiView detection system incl amplification kit.

Optimal results were typically obtained using the officially recommended protocol based on extended HIER in CC1 (92-104 min.), 16 min. incubation of the primary Ab, OptiView + amplification kit as detection system and BenchMark stainer platform. As mentioned above, no single cause in the data analysis have been identified to explain why the Ventana RTU system with two identical protocol settings can give these

significant divergent result ranging from passing with optimal mark to failing with borderline mark. NordiQC has informed Roche regarding the observation and they are actively monitoring customer feedback and working diligently to identify the root cause of this issue.

The relatively new Dako/Agilent RTU system for Omnis (prod. no. **GA785**) based on mAb clone OTI1A4 was the most successful assay, used by 41 laboratories on the Dako Omnis platform. When used as recommended, a pass rate of 100% was obtained, 84% being optimal. Minor adjustments in HIER time and/or incubation time of primary Ab were seen for the modified protocols, giving a pass rate of 89% (8 of 9) (see Table 4).

The Leica Bond RTU system based on mAb clone 5A4 (prod. no. **PA0831**) was used by 21 laboratories on a Bond platform. Nine laboratories used the vendor recommended protocol, giving a pass rate of 89% (8 of 9), 44% optimal. Minor adjustments in HIER time and/or incubation time of primary Ab were seen for the modified protocols, giving a pass rate of 83% (10 of 12) (see Table 4).

In concordance with previous assessments, concentrated Abs and RTU systems based on mAb clone ALK1 gave a low pass rate of only 7% (2 of 30). In most cases, the mAb clone ALK1 gave the expected staining reaction in the ALCL, but an insufficient (too weak or false negative) result in the lung adenocarcinoma with EML4-ALK translocation and the Merkel cell carcinoma. This indicates that mAb clone ALK1 is not fit for purpose demonstrating ALK fusion protein in EML-ALK translocated lung adenocarcinomas.

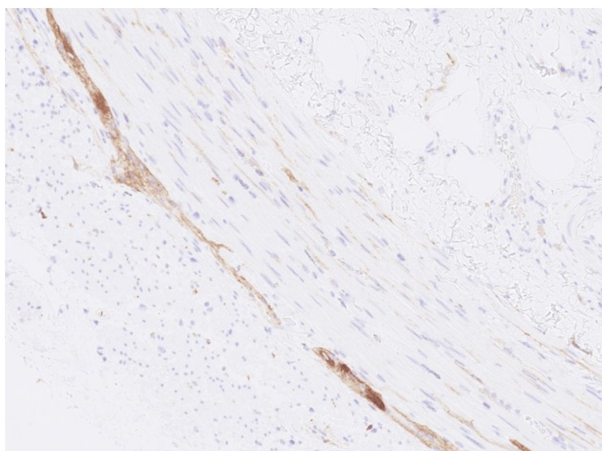


Fig. 1a
Optimal ALK staining reaction of axons and ganglion cells in appendix using the mAb clone OTI1A4 RTU (Dako/Agilent, GA785) by vendor recommended protocol settings. The ganglion cells of the myenteric plexus display a moderate, distinct cytoplasmic staining reaction, while the axons display a weak to moderate staining reaction.
Also compare with Figs. 2a - 4a, same protocol.

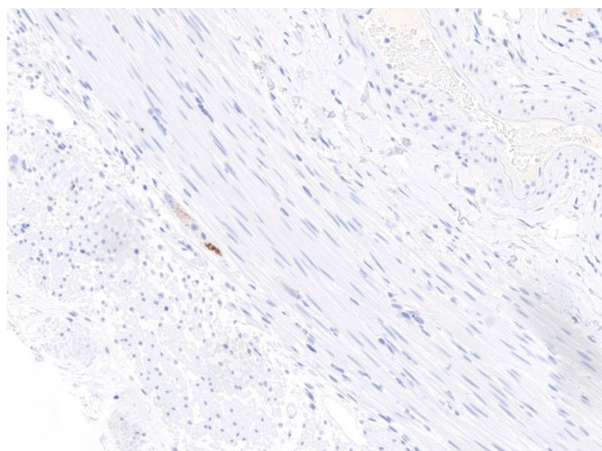


Fig. 1b
Insufficient ALK staining reaction of the appendix using same protocol as in Figs. 1b - 4b - same field as in Fig. 1a. Axons are unstained, and ganglion cells are only faintly positive. The protocol was based on the mAb clone 5A4 in a concentrated format, using HIER in an alkaline buffer and a 3-layer detection system. The protocol settings provided an overall too low level of analytical sensitivity.

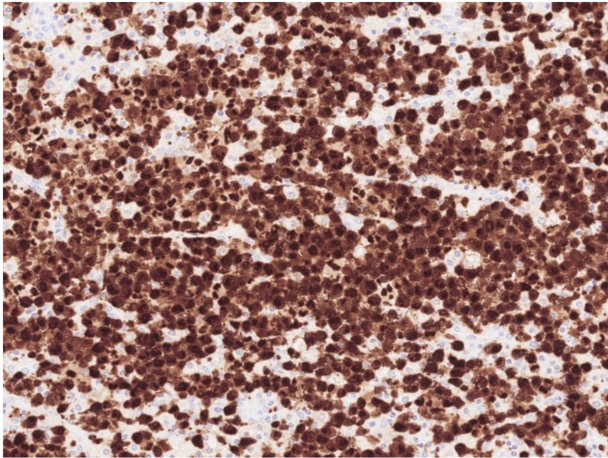


Fig. 2a
Optimal ALK staining reaction of the ALCL with ALK rearrangement using same protocol as in Fig. 1a. The neoplastic cells show an intense nuclear and cytoplasmic staining reaction.

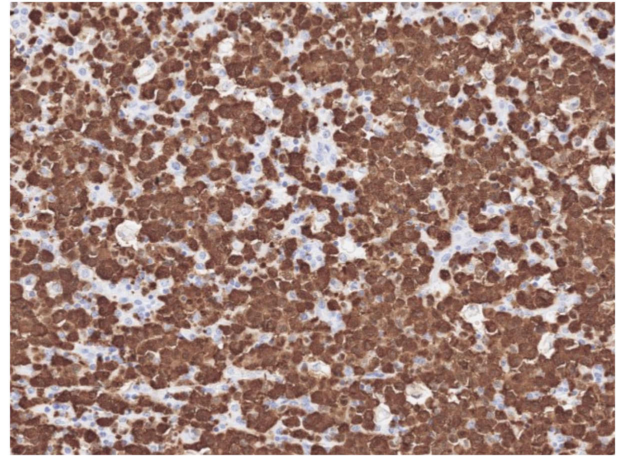


Fig. 2b
ALK staining reaction of the ALCL with ALK rearrangement using same protocol as in Fig. 1b. The neoplastic cells are demonstrated as expected.

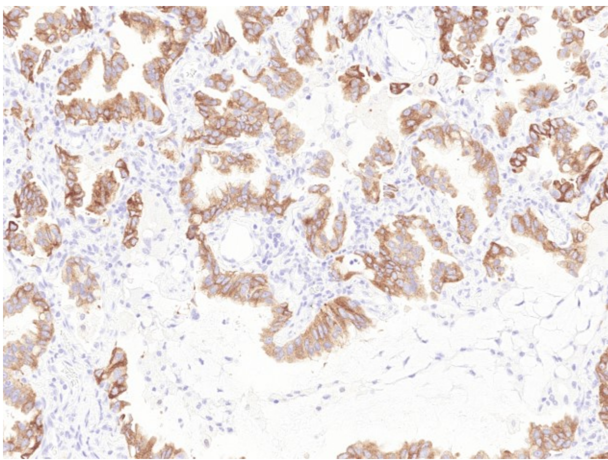


Fig. 3a
Optimal ALK staining reaction of the lung adenocarcinoma with ALK rearrangement using same protocol as in Figs. 1a – 2a. Most of the neoplastic cells show a moderate to strong granular cytoplasmic staining reaction. No background staining is seen.

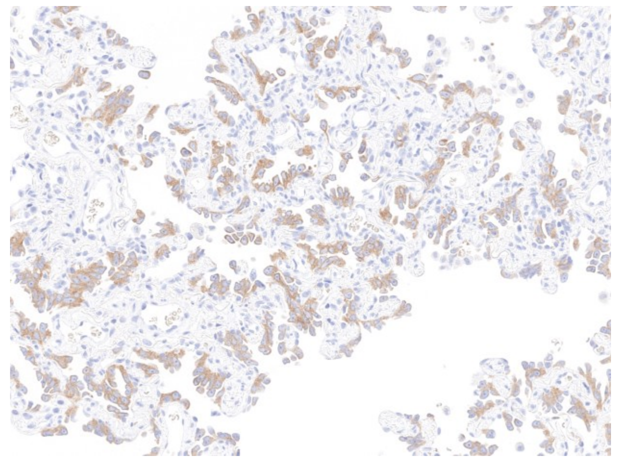


Fig. 3b
ALK staining reaction of the lung adenocarcinoma with ALK rearrangement using same protocol as in Figs. 1b – 2b - same field as in Fig. 3a. The neoplastic cells are demonstrated as expected. However, a weaker staining reaction is obtained.

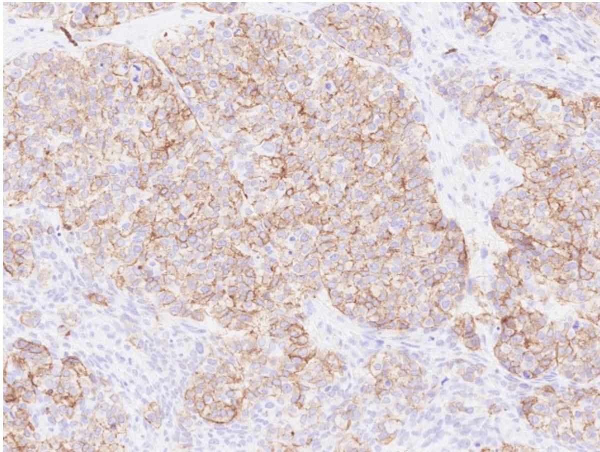


Fig. 4a
Optimal ALK staining reaction of the Merkel cell carcinoma using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a strong granular cytoplasmic staining reaction. No background staining is seen.

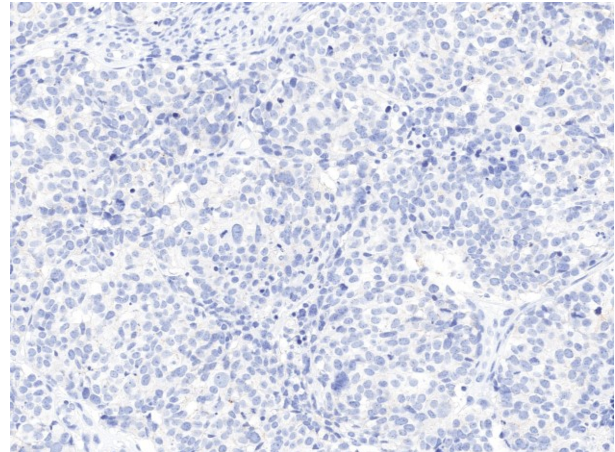


Fig. 4b
Insufficient ALK staining reaction of the Merkel cell carcinoma using same protocol as in Figs. 1b - 3b - same field as in Fig. 4a. The neoplastic cells are false negative. The amount of ALK protein in Merkel cell tumours is often on par with low level ALK expressing lung adenocarcinomas with EML4-ALK translocation and as such used as critical positive control for ALK to evaluate limit of demonstration for diagnostic importance.

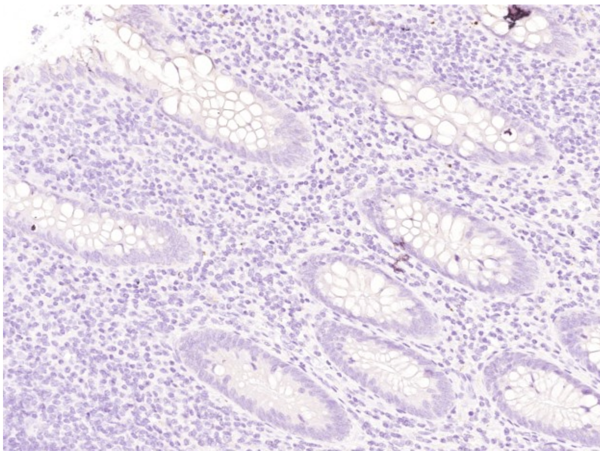


Fig. 5a
Optimal ALK staining reaction of lamina propria in appendix using the mAb clone D5F3 RTU (Ventana/Roche, 790-4794) by vendor recommended protocol settings. The epithelial cells are negative. Also compare with Fig. 6a, same protocol.

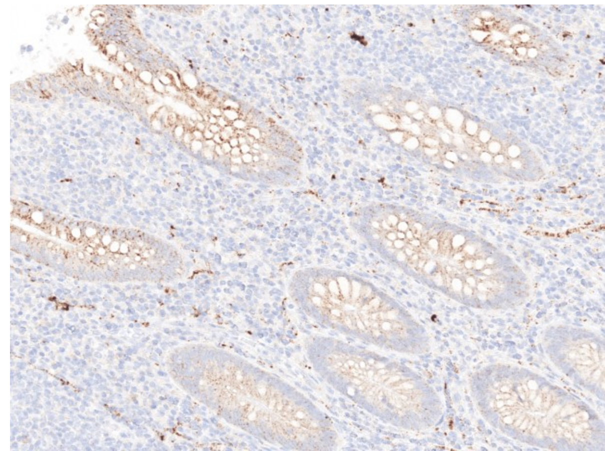


Fig. 5b
Insufficient ALK staining reaction of lamina propria in appendix using the mAb clone D5F3 RTU (Ventana/Roche, 790-4794) by vendor recommended protocol settings (same protocol as the optimal result in Fig. 5a). An aberrant staining reaction is seen in the epithelial cells, expected to be negative. Compare with Fig. 5a for optimal result (same field).

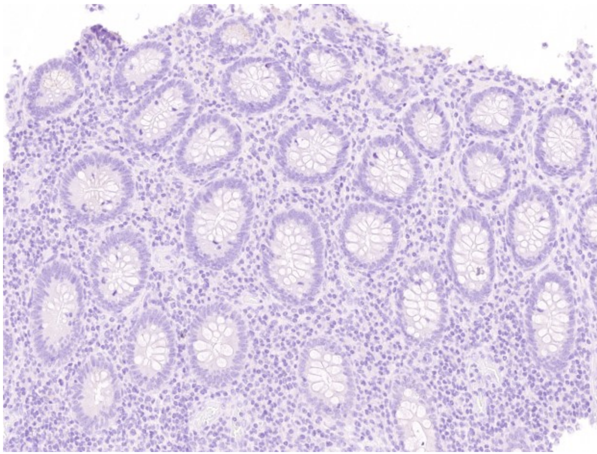


Fig. 6a
Optimal ALK staining reaction of an on-slide control tissue (colon/appendix) using same protocol as in Fig. 5a. The epithelial cells are negative as expected.

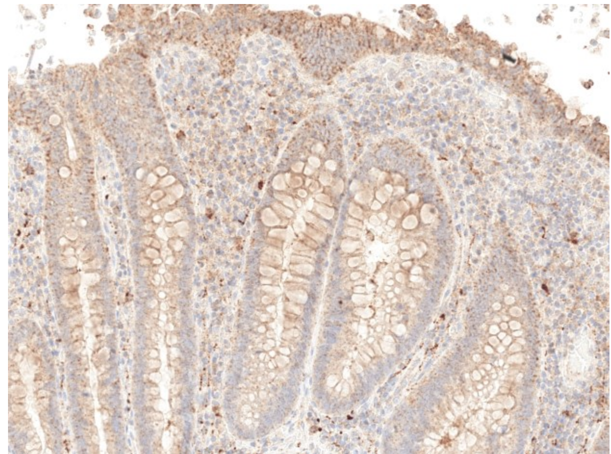


Fig. 6b
Insufficient ALK staining reaction of an on-slide control tissue (colon/appendix) using same protocol as in Fig. 5b. An aberrant staining reaction is seen in the epithelial cells and lymphocytes, expected to be negative.

HLK/LE/SN 04.04.2025