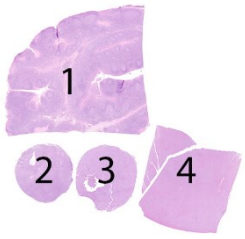
Assessment Run 75 2025
Cyclin D1
(CyD1)

Updated 06.03.26

Purpose
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CyD1, used for subclassification of lymphoproliferative disorders typically identifying mantle cell lymphomas (MCL) with chromosomal translocation (*11;14*). Relevant clinical tissues, both normal and neoplastic, were selected to represent a broad spectrum of antigen densities for CyD1 (see below).

Material
The slide to be stained for CyD1 comprised:



1. Tonsil, 2-3. Mantle cell lymphoma, 4. Chronic lymphatic leukemia (CLL)

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CyD1 staining as optimal included:

- A moderate to strong, distinct nuclear staining reaction of virtually all suprabasal squamous epithelial cells, scattered lymphocytes, macrophages and endothelial cells in the tonsil.
- An at least weak to moderate, distinct nuclear staining reaction of virtually all neoplastic cells in the two MCLs (tissue cores no. 2 and 3).
- An at least weak to moderate and distinct nuclear staining reaction of scattered endothelial cells in the CLL (tissue core no. 4).
- No staining or only a positive staining reaction in dispersed neoplastic cells in the CLL.
- No staining reaction in vast majority of germinal center lymphocytes, mantle zone B-cells and interfollicular lymphocytes in the tonsil.

A weak cytoplasmic staining reaction was accepted, provided that interpretation was not compromised.

KEY POINTS FOR CyD1 IMMUNOASSAYS
- The rmAb clones **EP12** and **SP4/SP4-R** were used by 97% of all participants either as concentrate or RTU format, providing a cumulated overall pass rate of 93%.
- The RTU systems **790-4508** (SP4-R), Ventana/Roche, **IR/GA083** (EP12), Dako/Agilent and **PA0046** (EP12), Leica Biosystems provided superior performance compared to laboratory developed assays based on concentrated formats of same clones.
- Tonsil is recommendable as positive and negative tissue control for CyD1.

Participation

Number of laboratories registered for CyD1, run 75	441
Number of laboratories returning slides	407 (92%)

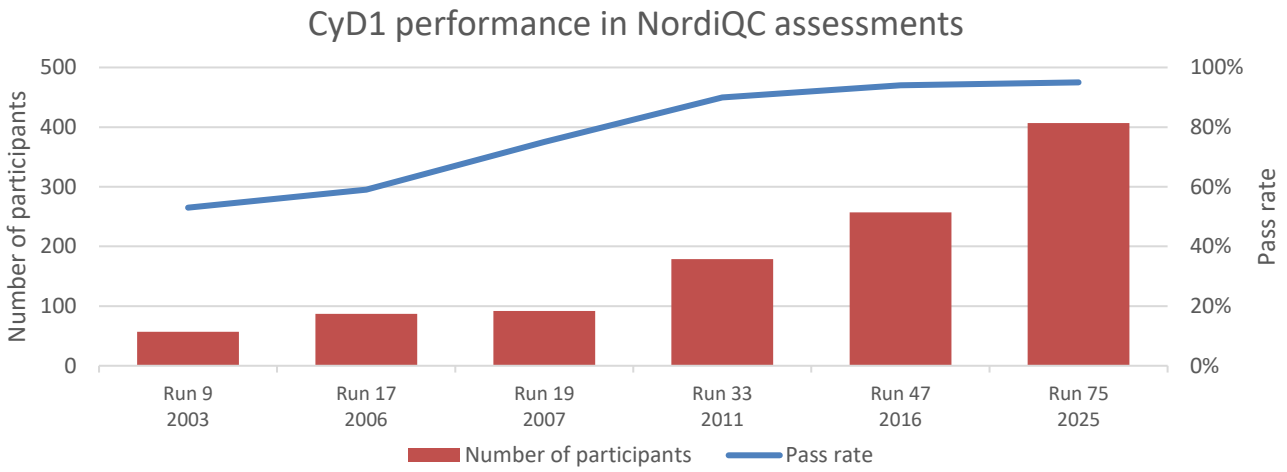
All slides returned after the assessment were assessed and participants received advice if the result was insufficient - data from these outcomes were not included in this report.

Results
407 laboratories participated in this assessment and 387 (95%) achieved a sufficient mark (optimal or good), see Table 1a (see page 3). Table 1b and 1c summarizes antibodies (Abs) used and assessment marks (see page 3 and 4).

The most frequent causes of insufficient staining reactions were:
- Excessive counterstaining compromising the interpretation.
- Inefficient HIER – too short HIER time.
- Less successful primary antibody.
- Unexplained.

Performance history
This was the sixth NordiQC assessment of CyD1. The pass rate was at the same level as in the previous run 47, 2016 (see Graph 1).

Graph 1. **Proportion of sufficient results for CyD1 in the six NordiQC runs performed**



Controls
Normal tonsil is recommended as positive and negative tissue control for demonstration of CyD1. Virtually all suprabasal squamous epithelial cells, scattered lymphocytes and endothelial cells must show a moderate to strong and distinct nuclear staining reaction. Within the germinal centers, dispersed germinal center macrophages must show an at least weak nuclear staining reaction serving as critical control of analytical sensitivity. In the mantle zones, T-zones and germinal centers, virtually all lymphocytes should be negative verifying the level of analytical specificity. Scattered lymphocytes can be positive, as CyD1 is a cell cycle associated protein. In addition, it should also be noted that the positive staining of endothelial cells can be a valuable internal positive tissue control for CyD1.

Conclusion
The rmAb clones **EP12** and **SP4/SP4-R** were used by 97% of all participants either as concentrate or RTU format, providing a cumulated overall pass rate of 93%. The two Abs gave optimal results both with 2-step and 3-step detection systems on virtually all applied IHC platforms underlining the high robustness of these two clones. The RTU systems based on either of these clones gave a pass rate of 94-100% and the high level was obtained both by laboratory modified or vendor recommended protocol settings. Tonsil was found to be a recommendable positive and negative tissue control to evaluate the required level of analytical sensitivity and specificity of the IHC assay for CyD1.

Table 1a. **Overall results for CyD1, run 75**

	n	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
Concentrated antibodies	52	23	19	9	1	81%	44%
Ready-To-Use antibodies	355	218	127	9	1	97%	61%
Total	407	241	146	18	2	-	
Proportion		59%	36%	4,5%	0,5%	95%	

1) Proportion of sufficient results (optimal or good).
2) Proportion of optimal results.

Table 1b. **Concentrated antibodies and assessment marks for CyD1, run 75**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **P2D11F11**	1	Leica Biosystems	0	0	0	1	-	-
rmAb clone **SP4**	16 10 4 2 1 1 1 1	Cell Marque Epredia/Thermo Fisher Biocare Zytomed Systems Diagnostic Biosystems ImmunoLine Invitrogen Nichirei	19	13	4	0	89%	53%
rmAb clone **EP12**	3 2 2 2	Cell Marque Dako/Agilent Diagnostic Biosystems Master Diagnostica	2	3	4	0	56%	22%
rmAb clone **BSR112**	1	Nordic Biosite	0	1	0	0	-	-
rmAb clone **GM009**	1	GeneBioSolution	0	0	1	0	-	-
rmAb clone **IHC452**	1	GenomeMe	1	0	0	0	-	-
rmAb clone **QR022**	1	Quartett	0	1	0	0	-	-
rmAb clone **ZR197**	1	Zeta Corporation	0	1	0	0	-	-
Unknown	1	Unknown	1	0	0	0	-	-
Total	52		23	19	9	1	-	
Proportion			44%	37%	17%	2%	81%	

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

Table 1c. **Ready-To-Use antibodies and assessment marks for CyD1, run 75**

Ready-To-Use antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
rmAb clone **EP12 IR083 (VRPS)**[3]	11	Dako/Agilent	7	4	0	0	100%	64%
rmAb clone **EP12 IR083 (LMPS)**[4]	16	Dako/Agilent	10	5	1	0	94%	63%
rmAb clone **EP12 GA083 (VRPS)**[3]	48	Dako/Agilent	38	9	1	0	98%	79%
mAb clone **EP12 GA083 (LMPS)**[4]	32	Dako/Agilent	21	11	0	0	100%	66%
rmAb clone **EP12 PA0046 (VRPS)**[3]	19	Leica Biosystems	6	12	1	0	95%	32%
mAb clone **EP12 PA0046 (LMPS)**[4]	15	Leica Biosystems	4	11	0	0	100%	27%
rmAb clone **EP12 MAD-000630**	2	Master Diagnostica	0	1	1	0	-	-
rmAb clone **EP12 8826-K250**	2	Sakura FineTek	1	1	0	0	-	-
rmAb clone **EP12 PME432**	1	Biocare Medical	0	1	0	0	-	-
rmAb clone **EP12 241R-47**	1	Cell Marque	0	0	1	0	-	-
rmAb clone **SP4-R 790-4508 (VRPS)**[3]	61	Ventana/Roche	38	22	0	1	98%	62%
rmAb clone **SP4-R 790-4508 (LMPS)**[4]	133	Ventana/Roche	86	43	4	0	97%	65%
rmAb clone **SP4 241R-17/18**	8	Cell Marque	3	5	0	0	100%	38%
rmAb clone **SP4 RM-9104-RQ**	1	Epredia/Thermo Fisher	0	1	0	0	-	-
rmAb clone **BP6076 BX50071**	1	Biolynx Biotechnology	1	0	0	0	-	-
rmAb clone **C12A7 CCR-1143**	1	Celnovte Biotechnology	1	0	0	0	-	-
rmAb clone **DY49967 492135**	1	Dakewe	1	0	0	0	-	-
rmAb clone **MXR056 RMA-1125**	1	Fuzhou Maixin	1	0	0	0	-	-
mAb clone **GM09 G221**	1	GeneTech	0	1	0	0	-	-
Total	355		218	127	9	1	-	
Proportion			61%	36%	3%	0%	97%	

1)

Proportion of sufficient results (optimal or good). (≥5 assessed protocols).
1) Proprtion of sufficient results (Suff.)
2) Proportion of Optimal Results (OR).
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 applied either on the vendor recommended platform(s), non-validated semi/fully automatic systems or used manually (≥5 assessed protocols)

Detailed analysis of CyD1, Run 75
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
rmAb clone **SP4**: Protocols with optimal results were based on Heat Induced Epitope Retrieval (HIER) using Cell Conditioning 1 (CC1, Ventana/Roche) (14/19), Bond Epitope Retrieval Solution 2 (BERS2, Leica Biosystems) (4/13) or Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako/Agilent) (1/1)* as retrieval buffer. The rmAb was typically diluted in the range of 1:25-1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings, 26 of 28 (93%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **EP12**: Protocols with optimal results were based based on HIER in an alkaline buffer using BERS2 (Leica Biosystems) (2/4) or CC1 (Ventana/Roche) (1/2) as retrieval buffer. The mAb was diluted in the range of 1:50-1:75 depending on the total sensitivity of the protocol employed. Using these protocol settings, 2 of 4 laboratories produced a sufficient staining result.

Table 2. **Proportion of optimal results for CyD1 for the most commonly used antibody concentrates 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 pH6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	BERS2 pH 9.0	BERS1 pH 6.0
rmAb clone **SP4**	-	-	1/1**	-	12/16 (75%)	-	4/11 (36%)	-
rmAb clone **EP12**	0/2	-	-	-	1/2	-	2/4	-

* 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 Classical, Link 48.
2) BenchMark GX, Ultra, Ultra Plus
3) Bond III/PRIME

Ready-To-Use antibodies and corresponding systems (≥5 protocols).

rmAb clone **EP12**, product no. **IR083**, Dako/Agilent, Autostainer Link:
Protocols with optimal results were typically based on HIER using TRS High pH (3-in-1) (efficient heating time 20 min. at 95-97°C), 20 min. incubation of the primary Ab and EnVision FLEX (K8002) as detection system. Using these protocol settings, 15 of 15 (100%) laboratories produced a sufficient staining result.
The product was used by 7 laboratories on a non-intended platform. These data are not included here.

rmAb clone **EP12**, product no. **GA083**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH (efficient heating time 30 min. at 97°C), 15-20 min. incubation of the primary Ab and EnVision FLEX+ (GV800+821) as detection system. Using these protocol settings, 56 of 57 (98%) laboratories produced a sufficient staining result. *The product was used by 1 laboratory on a non-intended platform. These data are not included here.*

rmAb clone **SP4-R**, product no. **790-4508**, Ventana/Roche, BenchMark GX/XT/Ultra/Ultra Plus:
Protocols with optimal results were typically based on HIER using CC1 (efficient heating time 32-72 min. at 95-100°C), 12-28 min. incubation of the primary Ab and OptiView (760-700) or UltraView (760-500) +/- amplification kit as detection system. Using these protocol settings, 109 of 111 (98%) laboratories produced a sufficient staining result.
The product was used by 1 laboratory on a non-intended platform. These data are not included here.

rmAb clone **EP12,** product no. **PA0046**, Leica Biosystems, BOND III/MAX/PRIME:
Protocols with optimal results were typically based on HIER using BERS2 (efficient heating time 20-30 min. at 97-104°C), 15-20 min. incubation of the primary Ab and Bond Polymer Refine Detection (DS9800) as detection system. Using these protocol settings, 28 of 29 (97%) laboratories produced a sufficient staining result.

Table 3 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 according 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 non-intended IHC stainers).

Table 3. **Proportion of sufficient and optimal results for CyD1 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS rmAb EP12 **IR083**	100% (11/11)	64% (7/11)	89% (8/9)	56% (5/9)
Dako Omnis rmAb EP12 **GA083**	98% (47/48)	79% (38/48)	100% (31/31)	65% (20/31)
VMS Ultra/Ultra PLUS/XT/GX mAb SP4-R **790-4508**	98% (60/61)	62% (38/61)	97% (128/132)	64% (85/132)
Leica BOND III/MAX/PRIME rmAb EP12 **PA0046**	95% (18/19)	32% (6/19)	100% (15/15)	27% (4/15)

* 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, detection kit – only protocols performed on the specified vendor IHC stainer are integrated.

Comments

In concordance with the previous NordiQC assessments for CyD1, the prevalent feature of an insufficient staining result was related to a generally too weak or false negative staining reaction of the cells expected to be demonstrated. This accounted for 60% (12/20) of the insufficient results. In 20%, a too weak staining reaction was seen in combination with an excessive counterstaining compromising the interpretation of the IHC signal for CyD1. The remaining insufficient results were characterized by false positive reactions and/or poor signal-to-noise ratio also affecting the interpretation of the specific CyD1 expression.
The results assessed as insufficient and evaluated as too week were typically characterized by showing a reduced staining reaction in both the neoplastic cells of the two mantle cell lymphomas but also in the dispersed lymphocytes, endothelial cells and especially the germinal center macrophages in the tonsil. This emphasizes the importance of using tonsil as critical positive tissue control for CyD1 and the ability to demonstrate CyD1 in the cells listed above ensuring an appropriate level of analytical sensitivity. In addition, it should also be noted that the positive staining of endothelial cells can be a valuable internal positive tissue control for CyD1.

The rmAb clones **EP12** and **SP4/SP4-R** were used by 97% of all participants either as concentrate or RTU format, providing a cumulated overall pass rate of 93% (see Tables 1b and 1c).

13% (52/407) of the participating laboratories used a concentrated Ab format within laboratory developed (LD) assays. This is significantly reduced compared to 43% in the latest assessment for CyD1, run 47 (2016). The overall pass rate for this group was 81% (44% optimal).
Optimal results were obtained by the rmAb clones **EP12**, **IHC452** and **SP4**. The rmAb clones EP12 and SP4 were most widely used. In this assessment the clone SP4 provided a superior performance compared to clone EP12 (see Table 1b). All protocols for the three Abs providing optimal staining results were based on efficient HIER in an alkaline buffer (e.g. CC1 from Ventana/Roche) in combination with either a 2- or 3-layer multimer/polymer-based detection system provided that the primary Ab
was carefully calibrated to the analytical sensitivity for the two different systems. For the 2-layer detection systems as UltraView, Ventana/Roche and Bond Refine, Leica Biosystems (Bond refine is a 2-layer system for rabbit primary antibodies as the linker only amplifies mouse primary antibodies) the average dilution value was 1:68 compared to 1:155 when a 3-layer detection system as OptiView, Ventana/Roche was applied.

87% (355/407) of all participating laboratories used a RTU format for the demonstration of CyD1. This is an increase compared to the previous run 47 (2016) in which 57% (146/257) of the participants applied a RTU format.

The Ventana/Roche RTU system **790-4508** based on the rmAb clone **SP4-R**, was used by 193 participants on the intended BenchMark IHC platform and provided an overall pass rate of 97% (188 of 193). The pass rate and proportion of optimal results was virtually identical for protocols based on the vendor recommended

settings and being modified by the laboratories (see Table 3). The present version of vendor recommended protocol settings for the Ventana/Roche RTU system suggest that both UltraView and OptiView can be applied as detection system. In this assessment OptiView was found to be superior to UltraView. Only focusing on the choice of detection system and not taking any other protocol steps as HIER and primary Ab incubation time into account, it was observed that protocols (n=87) based on OptiView (Prod. Id 760-700) as detection system gave an impressive pass rate of 100%, 95% being optimal. In contrast, protocols based on UltraView (Prod. Id 760-500) also provided a high pass rate of 96% (71/75) but only 39% was optimal (29/75). The application of OptiView improved the specific demonstration of CyD1 with a very high signal-to-noise ratio, whereas UltraView gave a less intense and less distinct nuclear staining reaction with a coexisting cytoplasmic reaction in plasma cells and serum/ background.

The Dako/Agilent RTU system **GA083** based on the rmAb clone **EP12** was used by 79 participants on the intended IHC platform Dako Omnis and obtained a pass rate of 98%, 79% being optimal when following the vendor protocol recommendations. The pass rate was 100% when modifying the protocol, but with a decreased level of 65% of optimal results (see Table 3). The modifications were mainly related to omission of rabbit linker or minor adjustments in incubation time in primary Ab.

In total, 27 participants used the Dako/Agilent RTU format **IR083** based on the rmAb clone **EP12**, of which 20 used the RTU format on the intended Autostainer Link 48+ platform, whereas 7 participants used the RTU format on a different stainer platform as Ventana BenchMark or Leica Bond III.
11 participants used the Dako/Agilent RTU system in compliance with vendor recommended protocol settings and a 100% pass rate was obtained.

The Leica Biosystems RTU system **PA0046** based on the rmAb clone **EP12** was used by 34 participants all performing the IHC assay on the intended platforms (BOND III, Bond PRIME and Bond MAX). Both the pass rate and proportion of optimal results were comparable by vendor recommended or modified protocol settings being applied (see Table 3). The overall pass rate was high and on par with the level obtained for the Ventana/Roche and Dako/Agilent RTU systems, but the proportion of optimal results was significantly reduced when compared to the two other RTU systems. In general, the intensity and number of positive cells demonstrated was decreased and not fully equivalent to the level expected. Protocols based on higher analytical sensitivity e.g. by prolonging HIER time and/or primary Ab incubation time did not improve the staining result.

This was the sixth assessment of CyD1 in NordiQC (see Graph 1). The pass rate of 95% was fully on par with the level seen in the previous run 47(2016) despite the number of participants has increased by nearly 60% from 257 in run 47 to 407 in this run 75. It is noteworthy to relate this level to the low pass rates of 53%-56% obtained in the first NordiQC runs for CyD1. These inferior results were largely attributable to the quality and affinity of the antibodies available at that time, such, as mAb clones **DCS6** and **P2D11F11**. The development of the two rmAb clones **SP4/SP4-R** and **EP12** has completely changed the scene and has been instrumental for the improved quality of IHC assays for CyD1. In addition, the access to and extensive use of well performing RTU systems from the main IHC system providers has been essential to obtain and maintain the high quality for CyD1 IHC. In this context, it was also encouraging to see that many new IHC systems based on RTU formats on new Ab clones were introduced e.g. from Biolynx, Celnovte, Dakewe and Maixin and obtained optimal results (see Table 1c).

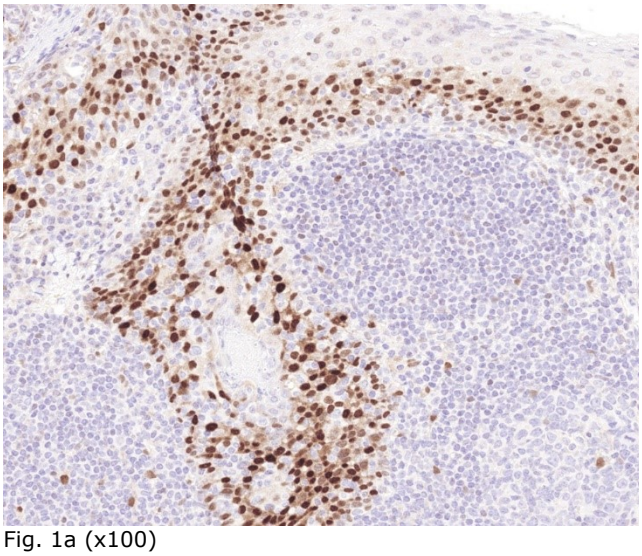


Fig. 1a (x100)
Optimal CyD1 staining result of the tonsil using the RTU format 790-4508 (Ventana/Roche) based on rmAb clone SP4-R on BenchMark Ultra, following the protocol recommendations given by the vendor using OptiView as detection system – same protocol used in Figs. 1a – 4a.
Virtually all suprabasal epithelial cells show a moderate to strong nuclear staining reaction. Most intermediate and superficial cells are negative or only showing a weak staining reaction.

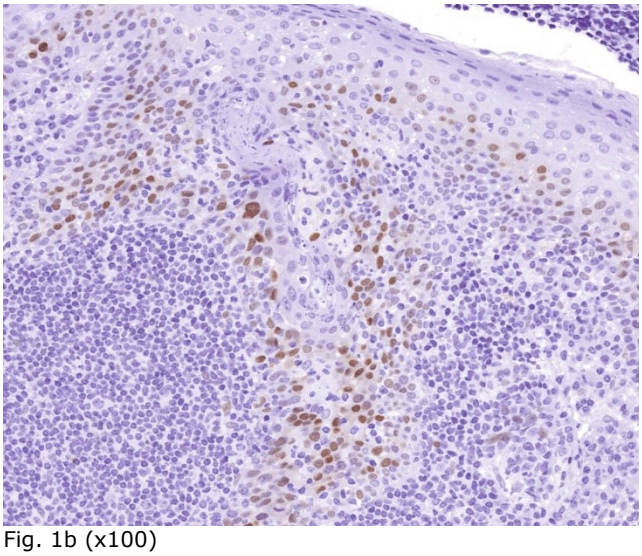


Fig. 1b (x100)
Insufficient staining result of the tonsil using the rmAb clone EP12 as concentrate on the Dako Omnis (Dako/Agilent) with HIER in TRS High pH and FLEX+ as detection system – same protocol used in Figs. 1b – 4b.
Despite selecting protocol settings expected to provide a high analytical sensitivity, the result was not as expected. The proportion and staining intensity of the suprabasal epithelial cells is reduced compared to level expected and seen in Fig. 1a.
A too low conc. of the primary Ab in combination with excessive counterstaining caused the inferior result.

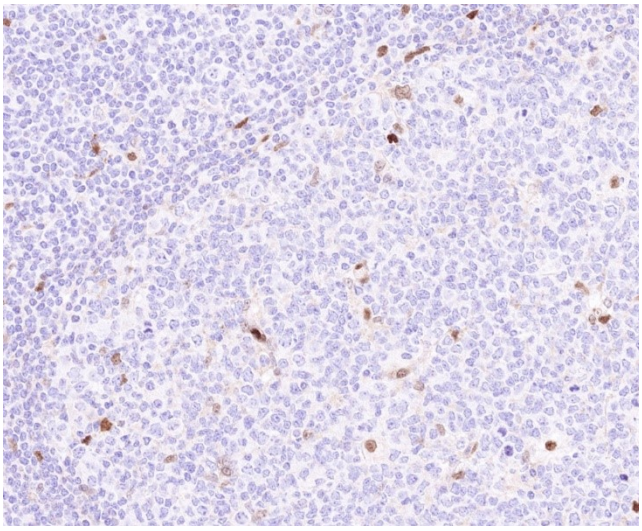


Fig. 2a (x200)
Optimal staining result of the tonsil - Same protocol as in Fig. 1a.
Dispersed germinal center macrophages and endothelial cells show a weak to moderate and distinct nuclear staining reaction. Only scattered lymphocytes are positive while most being completely negative.

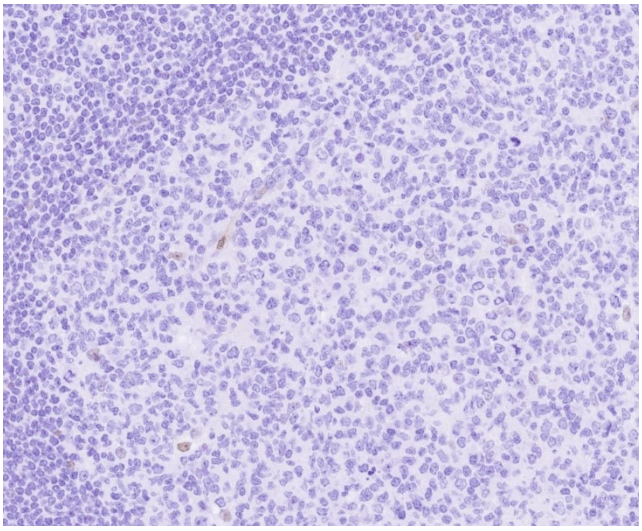


Fig. 2b (x200)
Insufficient staining result of the tonsil using same protocol as in Fig. 1b.
All germinal center macrophages and endothelial cells are virtually negative – only few cells show a faint and equivocal nuclear staining reaction.

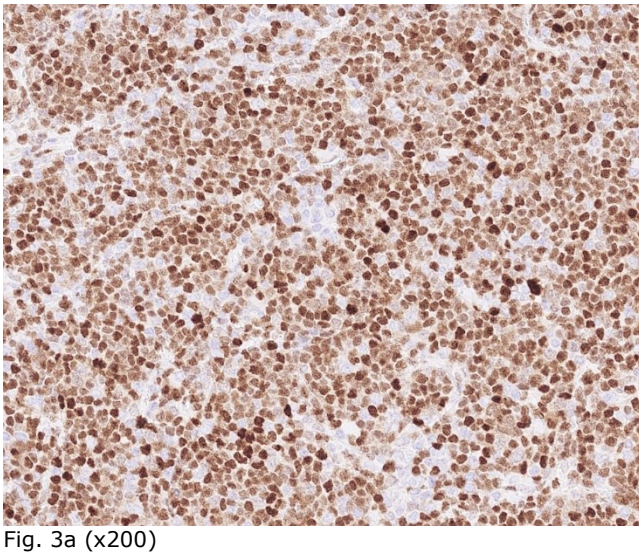


Fig. 3a (x200)
Optimal staining result for CyD1 of the mantle cell lymphoma, tissue core no. 2, same protocol used in Figs. 1a - 2a.
All neoplastic cells display a moderate to strong, distinct nuclear staining reaction.

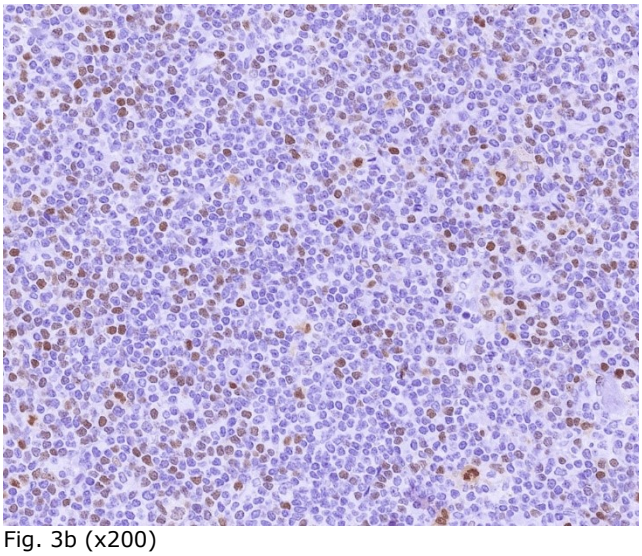


Fig. 3b (x200)
Insufficient staining result for CyD1 of the mantle cell lymphoma, tissue core no. 2, same protocol used in Figs. 1b – 2b.
Only few neoplastic cells show a distinct nuclear staining reaction.

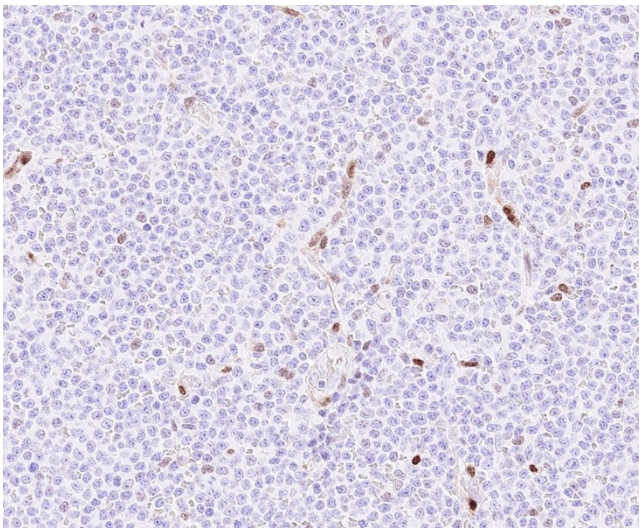


Fig. 4a (x100)
Optimal staining result for CyD1 of the CLL, tissue core no. 4, using same protocol as in Figs. 1a - 3a.
The neoplastic cells are negative, while mainly endothelial cells show a moderate and distinct nuclear staining reaction, serving as internal positive tissue control.

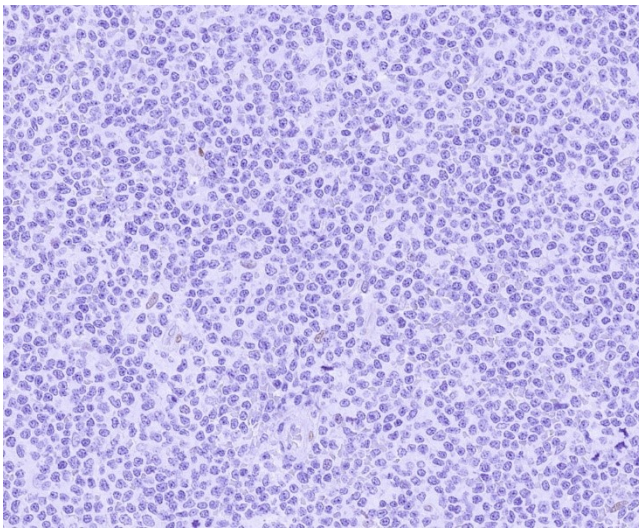


Fig. 4b (x100)
Staining result for CyD1 of the CLL, tissue core no. 4, using same protocol as in Figs. 1b – 3b.
Both the neoplastic cells and endothelial cells are negative.

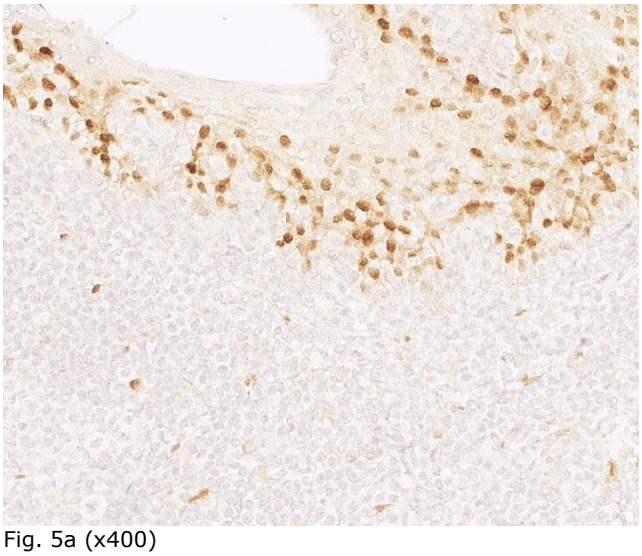


Fig. 5a (x400)
Insufficient staining result for CyD1 of the tonsil. The protocol was based on a RTU format of the rmAb clone EP12 in combination with HIER in a pressure cooker using an alkaline EDTA buffer.
The tissue morphology and crispness of the IHC signal is impaired. In addition, the nuclear counterstaining is weak overall complicating the interpretation of the staining reaction.
Excessive HIER most likely caused this insufficient result.
Also see Fig. 5b – same protocol.

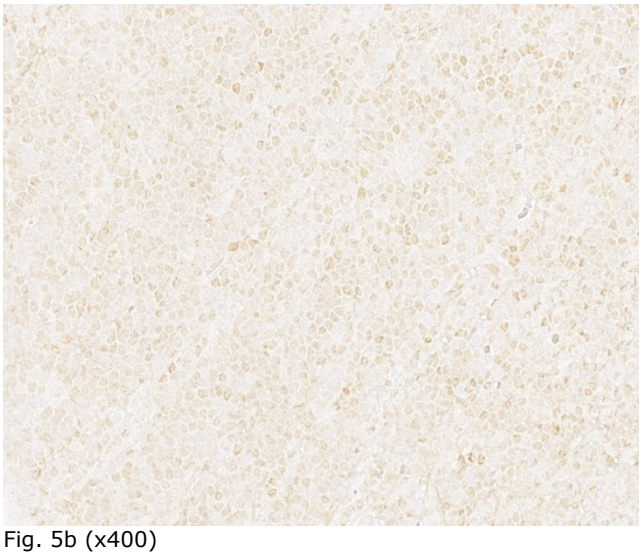


Fig. 5b (x400)
Insufficient staining result for CyD1 of the mantle cell lymphoma, tissue core no. 2, same protocol used in Fig. 5b.
The combination of weak IHC staining reaction for CyD1, excessive HIER and weak nuclear counterstaining obscures the interpretation of the CyD1 staining reaction.

SN/LE/RR 15.12.2025

Version	Description of change and reason	Date	Authorized by
2	Table 1c has been updated as wrong numbers was written in version 1.	06.03.26	SN/LE/RR