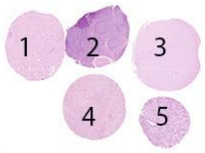
Assessment Run 60 2020
CD68

Purpose
Evaluation of the technical performance and level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CD68. Intended purpose in general pathology to identify macrophages, identification of myeloid/histiocytic sarcoma and to differentiate macrophages from epithelial tumour cells in lung pathology for e.g. accurate PD-L1 TPS status. Relevant clinical tissues, both normal and neoplastic, were selected for a broad spectrum of antigen densities for CD68 (see below).

Material
The slide to be stained for CD68 comprised:



1. Liver, 2. Tonsil, 3. Brain, 4. Histiocytic sarcoma, 5. Lung adenocarcinoma

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD68 staining as optimal included:

- A strong and distinct cytoplasmic staining reaction of the germinal centre macrophages in the tonsil.
- A moderate to strong cytoplasmic staining reaction of the macrophages in the lung adenocarcinoma and interfollicular zones of the tonsil and in the Kupffer cells of the liver.
- An at least weak to moderate cytoplasmic staining reaction of the microglial cells in the brain.
- An at least moderate cytoplasmic staining reaction in virtually all the neoplastic cells of the histiocytic sarcoma.
- No staining reaction in the liver cells, or neoplastic cells of the lung adenocarcinoma and no cytoplasmic staining in the epithelial cells in the tonsil.

In this test normal brain was an efficient positive control for CD68, as all the laboratories obtaining a sufficient result could demonstrate CD68 in the microglial cells. However, in order to evaluate the specificity and a proper signal-to-noise ratio it is advisable also to use tonsil as control, in which the germinal centre B-cells must be negative. Using the mAb clone KP1 a weak to moderate cytoplasmic staining in the squamous epithelial cells should be accepted.

Participation

Number of laboratories registered for CD68, run 60	364
Number of laboratories returning slides	330 (91%)

The number of laboratories returning slides has decreased in this run 60 compared to previous assessments, due to the Covid-19 pandemic and associated postal delays. All slides returned after the assessment were assessed, and received advice if the result being insufficient, but data was not included in this report.

During the assessment a limited number of participants have experienced issues with the circulated NordiQC slides, providing a partial or entire aberrant/false negative staining result in some cases. During assessment, this observation was taken into account and for CD68, seven slides were potentially affected. Six were assessed and one excluded. If performance was characterized by uneven staining or a completely false negative result that could be related to the quality of the slide and not the protocol submitted, this was commented in the individual assessment feed-back.

Results
329 laboratories participated in this assessment. 274 (83%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and the assessment marks given (see page 3).

The most frequent causes of insufficient staining reactions were:
- Too low concentration of the primary antibody
- Insufficient Heat Induced Epitope Retrieval (HIER) (too low temperature and/or too short efficient heating time)
- Proteolytic pre-treatment or omission of epitope retrieval
- Unexplained technical issues

Performance history
This was the fourth NordiQC assessment of CD68. Despite a large increase of new participants since the latest assessment, the overall pass rate was almost the same as the previous run for CD68 (see Table 2).

Table 2. **Proportion of sufficient results for CD68 in the four NordiQC runs performed**

	Run 11 2004	Run 26 2009	Run 31 2011	Run 60 2020
Participants, n=	64	128	157	329
Sufficient results	73%	70%	82%	83%

Conclusion
The mAb clones **PG-M1**, **KP1**, **514H12** and rmAb clone **GR021** could all be used to provide an optimal result for CD68. **PG-M1** was most widely used and gave the highest proportion of optimal results. Irrespectively of clone HIER (preferable in an alkaline buffer) is mandatory to obtain an optimal result. Brain and tonsil are recommended as positive and negative tissue controls. In the brain, the vast majority of microglial cells must show an at least weak to moderate but distinct cytoplasmic staining reaction. In the tonsil the germinal centre macrophages must show a strong cytoplasmic staioning reaction, but of central importance virtually all interfollicular macrophages must show a moderate to strong cytoplasmic staining reaction, while the germinal centre B-cells should be negative.

Table 1. **Antibodies and assessment marks for CD68, run 60**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	OR[2]
mAb clone **514H12**	4	Leica/Novocastra	1	2	-	1	-	-
mAb clone **KP1**	34 3 2 3 1 1 1 1	Dako/Agilent Biocare Medical Leica Biosystems Thermo Scientific BioGenex Neomarkers Novocastra Zeta Corporation	4	30	7	5	74%	9%
mAb clone **PG-M1**	81 4 1	Dako/Agilent Diagnostic Biosystems Neo Markers	53	22	6	5	87%	62%
rmAb clone **BP6036**	1	Bailing Biotechnology	-	1	-	-	-	-
Ready-To-Use antibodies							Suff.[1]	OR.[2]
mAb clone **KP1, 168M-98**	1	Cell Marque	-	1	-	-	-	-
mAb clone **514H12, 002097QD**	1	Master Diagnostica	-	1	-	-	-	-
mAb clone **PG-M1, PDM065**	1	BioSystems	-	1	-	-	-	-
mAb clone **KP1, PM 033 AA**	1	Biocare Medical	-	1	-	-	-	-
mAb clone **KP1, MAD002097**	1	Master Diagnostica	-	1	-	-	-	-
mAb clone **514H12, PA0273 (VRPS)[3]**	12	Leica Biosystems	3	8	1	-	92%	25%
mAb clone **514H12, PA0273 (LMPS)[4]**	5	Leica Biosystems	-	3	1	1	80%	-
mAb clone **PG-M1, GA613 (VRPS)[3]**	8	Agilent/Dako	5	3	-	-	100%	63%
mAb clone **PG-M1, GA613 (LMPS)[4]**	1	Agilent/Dako	-	-	-	1	-	-
mAb clone **PG-M1, IR/IS613 (VRPS)[3]**	12	Agilent/Dako	12	-	-	-	100%	100%
mAb clone **PG-M1, IR/IS613 (LMPS)[4]**	31	Agilent/Dako	18	10	2	-	92%	59%
mAb clone **KP1, GA609 (VRPS)[3]**	19	Agilent/Dako	-	18	1	-	95%	-
mAb clone **KP1, GA609 (LMPS)[4]**	9	Agilent/Dako	1	4	4	-	56%	11%
mAb clone **KP1, IR/IS609 (VRPS)[3]**	6	Agilent/Dako	1	3	2	-	67%	17%
mAb clone **KP1, IR/IS609 (LMPS)[4]**	3	Agilent/Dako	1	1	1	-	-	-
mAb clone **KP1, 790-2931 (VRPS)[3]**	2	Roche/Ventana	-	2	-	-	-	-
mAb clone **KP1, 790-2931 (LMPS)[4]**	76	Roche/Ventana	4	55	10	7	78%	5%
rmAb clone **GR021, 8275 (VRPS)[3]**	3	Sakura Finetek	3	-	-	-	-	-
Total	329		106	168	35	20		
Proportion			32%	51%	11%	6%	83%	

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).
5) Ab terminated by vendor.

Detailed analysis of CD68, Run 60
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **PG-M1**: The protocols giving an optimal result were typically based on HIER using either Target Retrieval Solution pH 9 (TRS pH 9) (3-in-1) (Dako/Agilent) (2/8)*, TRS pH 9 (Dako) (4/10), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (9/11), Bond Epitope Retrieval Solution 1 (BERS1, Leica) (4/5) or Cell Conditioning 1 (CC1, Ventana) (29/43) as the retrieval buffer. The mAb was typically diluted in the range of 1:50–200 depending on the total sensitivity of the protocol employed. Using these protocol settings 57 out of 63 (90%) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **KP1**: The protocols giving an optimal result were all based on HIER using TRS pH 9 (3-in-1) (Dako/Agilent) (1/5), CC1 (Ventana) (2/27) or BERS2 (Leica) (1/2) as the retrieval buffer. The mAb was typically diluted in the range of 1:500-10.000 depending on the total sensitivity of the protocol employed. Using these protocol settings 19/27 (70%) laboratories produced a sufficient staining (optimal or good).

mAb clone **514H12**: One protocol giving an optimal result was based on BERS1 (Leica) (1/1) as the retrieval buffer. The mAb was diluted 1:100.

Table 3. **Proportion of optimal results for CD68 for the most commonly used antibodies as concentrate on the 4 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Dako Omnis**		**Ventana BenchMark GX / XT / Ultra**		**Leica Bond III / Max**	
	TRS pH 9.0	TRS pH 6.1	TRS pH 9.0	TRS pH 6.1	CC1 pH 8.5	CC2 pH 6.0	ER2 pH 9.0	ER1 pH 6.0
mAb **514H12**	-	-	-	-	0/2	-	0/1	1/1
mAb **KP1**	1/5	-	0/1	0/1	2/27	0/2	1/2	0/1
mAb **PG-M1**	2/8	-	4/10	-	29/43	1/1	9/11	4/5

* 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.

Ready-To-Use antibodies and corresponding systems
mAb clone **KP1**, product no. **790-2931**, Roche/Ventana, Ventana Benchmark:
Protocols with optimal results were typically based on HIER in CC1 (efficient heating time 24-64 min. at 90-100°C), 8-12 min. incubation of the primary Ab and UltraView (760-500) or OptiView (760-700) as detection systems. Using these protocol settings, 12 of 13 (92%) laboratories produced a sufficient result. Applying VRPS, 2 of 2 laboratories produced a sufficient result assessed as good (see Table 4).

mAb clone **KP1**, product no. **IS/IR609**, Dako/Agilent, Autostainer:
Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1) (efficient heating time 10-20 min. at 96-97°C), 20 min. incubation of the primary Ab and EnVision Flex (K8000) with or without linker as detection system. Using these protocol settings, 6 of 8 (75%) laboratories produced a sufficient result. Applying VRPS, the proportion of sufficient results was 67% (4/6) and one of the laboratories produced an optimal staining result (see Table 4).

mAb clone **KP1**, product no. **GA609**, Dako/Agilent, Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH 9 (GV804) (efficient heating time 20-30 min. at 97°C) and 15-20 min. incubation of the primary Ab and EnVision FLEX (GV800) as detection system. Using these protocol settings, 23 of 25 (92%) laboratories produced a sufficient staining result. Applying VRPS, the proportion of sufficient results was 95% (18/19), no optimal results (see Table 4).

mAb clone **PG-M1**, product no. **IS/IR613**, Dako/Agilent , Autostainer:
Protocols with optimal results were typically based on HIER using TRS pH 9 (3-in-1) (efficient heating time 15-20 min. at 96-97°C), 15-30 min. incubation of the primary Ab and EnVision Flex (K8000) as detection system. Using these protocol settings, 16 of 19 (84%) laboratories produced a sufficient result. Applying VRPS, the proportion of sufficient results was 100% (12/12), all optimal (see Table 4).

mAb clone **PG-M1**, product no. **GA613**, Dako/Agilent , Omnis:
Protocols with optimal results were typically based on HIER using TRS High pH 9 (GV804) (efficient heating time 30 min. at 97°C) and 20-25 min. incubation of the primary Ab and EnVision FLEX (GV800) as

detection system. Using these protocol settings, 8 of 8 (100%) laboratories produced a sufficient staining result. Applying VRPS, the proportion of sufficient results was 100% (8/8) and 63% (5/8) of the laboratories produced an optimal staining result (see Table 4).

mAb clone **514H12**, product no. **PA0273**, Leica Biosystems, Leica Bond:
Protocols with optimal results were typically based on HIER using BERS2 (efficient heating time 20-30 min. at 100°C), 15-20 min. incubation of the primary Ab and BOND Refine (DS9800) as the detection system. Using these protocol settings, 11 of 15 laboratories (73%) produced a sufficient result. Applying VRPS, the proportion of sufficient results was 83% (10/12) and 25% (3/12) optimal (see Table 4).

Table 4 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥5 asessed 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.

Table 4. **Proportion of sufficient and optimal results for CD68 for the most commonly used RTU IHC systems**

RTU systems	**Recommended protocol settings***		**Laboratory modified protocol settings****	
	Sufficient	Optimal	Sufficient	Optimal
Dako AS mAb PG-M1 **IS/IR613**	100% (12/12)	100% (12/12)	88% (7/8)	50% (4/8)
Dako Omnis mAb PG-M1 **GA613**	100% (8/8)	63% (5/8)	(0/1)	(0/1)
Dako AS mAb KP1 **IS/IR609**	67% (4/6)	17% (1/6)	(2/2)	(1/2)
Dako Omnis mAb KP1 **GA609**	95% (18/19)	0% (0/19)	50% (4/8)	11% (1/8)
VMS Ultra/XT mAb KP1 **790-2931**	(2/2)	(0/2)	78% (59/76)	5% (4/76)
Leica BOND MAX/III mAb **514H12** **PA0273**	83% (10/12)	25% (3/12)	60% (3/5)	(0/5)

* 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 and in concordance with the previous NordiQC CD68 assessments, the prevalent feature of an insufficient staining result was a too weak or completely false negative staining reaction of cells expected to be demonstrated. This pattern was seen in 75% (41 of 55) of the insufficient results. Virtually all the participating laboratories were able to demonstrate CD68 in the germinal centre macrophages of the lymphatic secondary follicles and the neoplastic cells in the histiocytic sarcoma, whereas the demonstration of CD68 in the interfollicular macrophages and in particular the microglial cells of the brain was more challenging and only seen with appropriate protocol settings, e.g., a correct titre of the mAb clones PG-M1, KP1 and 514H12 and the use of HIER.

Enzymatic pre-treatment was used by 7 of the laboratories as epitope retrieval. One laboratory was able to obtain an optimal result, but in general proteolysis was seen to give not only a reduced analytical sensitivity (as the microglial cells only showed a too weak or complete negative staining reaction) but also induced an impaired morphology due to digestion of the fragile cell membranes (see figs. 8a and 8b) in cells of interest.

42% (138 of 330) of the laboratories used concentrated Ab formats within laboratory developed (LD) assays for CD68. The mAb PG-M1 was the most widely used Ab and could be used to obtain optimal staining results on all 4 of the main IHC platforms as shown in Table 3. Used within a LD assay, the mAb PG-M1 gave an overall pass rate of 87% (75/86) and 62% (53/86) optimal.
HIER, preferable in an alkaline buffer, in combination with a careful calibration of the primary Ab seem to be the most critical parameters for sufficient and optimal results. 3 of the 11 insufficient results was due to

technical issues, but not fully evident to identify the root cause as being glass quality, stainer or protocol issues.

46 laboratories used the mAb clone KP1, which gave an overall pass rate of 74% (34/46) but only 9% (4/46) being optimal. The optimal results were observed on 3 out of the 4 main IHC platforms (see Table 3), all using HIER in an alkaline buffer and a high dilution of 1:500-10.000. The mAb clone KP1 is less specific and provides a reduced selectivity regarding reaction pattern compared to the clone PG-M1 and hereby induce a challenge to optimize and calibrate the protocols e.g. to identify macrophages without having an extensive cross reaction in e.g. epithelial cells. This challenge was confirmed in the results observed as out of the 46 participants using the KP1 as a concentrate 15 slides were marked weak and 11 with a poor-signal-to-noise ratio.

4 laboratories used the mAb clone 514H12 from Leica/Novocastra on either the Ventana benchmark or Leica Bond platform. 3 produced a sufficient result. The antibody was used in low dilutions in the range of 1:40-100 and primarily with HIER in an alkaline buffer. One protocol with an optimal result was based on HIER for 30 min. in a low pH buffer and with an Ab incubation time of 30 min. (see Tabel 3).

Overall, Ready-To-Use (RTU) systems or RTU formats were used by 58% (192/330) of the participants. The Ventana RTU system based on mAb clone KP1 (790-2931) was used by 78 laboratories. Only 2 laboratories followed the vendor protocol recommendations giving 2 sufficient results but no optimal. The 76 laboratories that used a modified protocol obtained a total of 78% (59/76) sufficient results but with low proportion of 5% optimal results. The 4 protocols giving an optimal result were all based on OptiView +/- amplification kit as detection system, HIER in CC1 for 24-64 min. and a short Ab incubation time of 8-12 min. The short Ab incubation time seems crucial to reduce background reaction and/or false positive staining and avoiding loss of reactivity in the microglial cells of the brain with low level CD68 expression.

The Dako/Agilent RTU systems GA609 (Omnis) and IR/IS609 IR/IS609 (Autostainer) based on mAb clone KP1 were used by 28 and 9 laboratories, respectively. Overall a pass rate of 82% was seen, but only 8% being optimal (3/37). As listed in Tables 1 and 4, no significant difference of pass rates and proportion of optimal results were observed for VRPS versus LMPS. All of the participants used EnVision Flex as detection system (2 with linker), and HIER in an alkaline buffer for 20-30 min. Of particular notice the chromogene incubation time of 10 min. instead of 5 min. seemed to have a positive effect on all protocols based on this RTU format. Overall the results were characterized by too weak and reduced proportion of cells expected to be demonstrated as e.g. the microglial cells in the brain and/or the interfollicular makrophages in the tonsil.

43 laboratories used the Dako/Agilent RTU system IS/IR613 based on the mAb clone PG-M1 for Autostainer and 93% (40/43) received a sufficient mark, 70% (30/43) optimal. Following the VRPS 100% (12/12) of the laboratories scored an optimal result making this the most successful of the RTU systems. 31 laboratories modified the protocol and 91% (28/31) produced a sufficient result, 59% (18/31) optimal. 22 laboratories applied this clone to other platforms than the autostainer.

9 laboratories used the Dako/Agilent RTU system GA613 also based on the mAb clone PG-M1 for Omnis and in total 89% (8/9) received a sufficient result. Following the VRPS 100% (8/8) produced a sufficient result, 63% optimal. On the Omnis it seemed crucial to use the Envision Flex+ protocol to be able to demonstrate the critical structures with low level CD68 expression in this assessment – the only protocol applied with omission of linker gave an insuffcient result.

In generel the Envision Flex+ protocol on the Dako Omnis was very succesfull demonstrating the critical structures with the low level CD68 expression in both concentrated and RTU formats. In total 62 participants used the Dako Omnis and 26 with Envision Flex+ mouse linker and all received a sufficient result, 62% (16/26) optimal. 36 of the Omnis users did not use the Flex+ protocol and for this group a pass rate of 81% was seen, but only 3% optimal.

17 laboratories used the Leica Biosystems RTU system based on mAb clone 514H12 and overall 82% (14/17) with a sufficient result, 18% (3/17) optimal. Applying the RTU accordingly to the VRPS the pass rate was 83% (10/12), 25% optimal (3 of 12). Insufficient results were primarily due to the use of low pH buffer for HIER.

In this assessment 3 laboratories used the the Sakura Finetek RTU system based on the rmAb clone GR021. All 3 laboratories used the system in compliance with the VRPS on the Sakura Genie platform with 100% (3/3) optimal results, using an alkaline buffer for HIER for 45 min. and 30 min. Ab incubation time.

This was the fourth NordiQC assessment for CD68. The number of participants were almost doubled compared to the previous run 31 (see Table 2) but with similar pass-rates in these two runs. In this assessment an extended use of RTU formats/systems was observed. In run 31 21% (33/157 laboratories) used RTU formats/systems, compared to 58% (192/330) in this run. 38% (62/192) laboratories followed the VRPS with a total pass rate of 93% (58/62), compared to a pass-rate of 79% (102/130) for laboratories using LMPS.

Controls

Brain and tonsil are recommended as positive and negative tissue controls. In the brain, the microglial cells must show an at least weak but distinct cytoplasmic staining reaction, while the background must be negative. In the tonsil the interfollicular macrophages must show a moderate to strong cytoplasmic staining reaction, while the germinal centre B-cells should be negative.

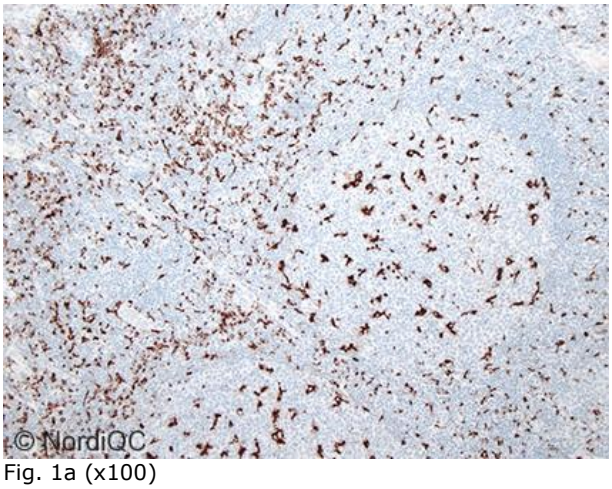


Fig. 1a (x100)
Optimal staining reaction for CD68 of the tonsil using the mAb clone PG-M1 as RTU (IS613), HIER in an alkaline buffer (TRS pH 3-in-1) and a polymer-based detection system (FLEX, Dako/Agilent) - same protocol used in Figs. 2a - 5a. The germinal center and interfollicular macrophages show a moderate to strong distinct cytoplasmic staining reaction, with no background or staining of the germinal centre B-cells - compare with Fig. 1b.

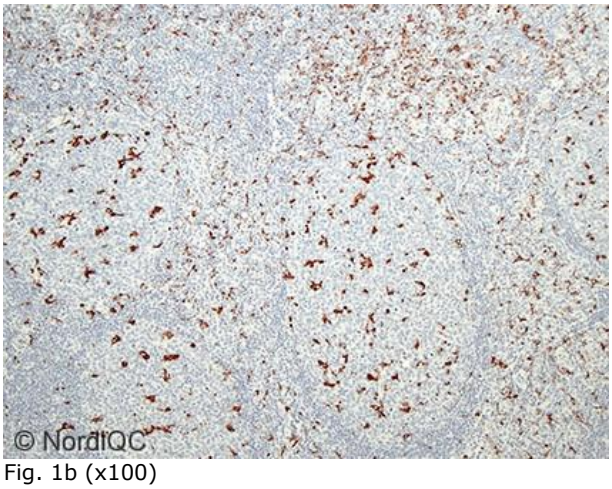


Fig. 1b (x100)
Staining reaction for CD68 of the tonsil using the mAb clone PG-M1 as RTU (IS613), HIER in an low pH buffer (TRS pH 6) and a polymer-based detection system (FLEX, Dako/Agilent) - same protocol used in Figs. 2b – 5b. Both the intensity of the staining reaction, and the amount of the interfollicular macrophages are reduced - compare with Fig. 1a.
However also compare with Figs. 1 – 5b, same protocol.

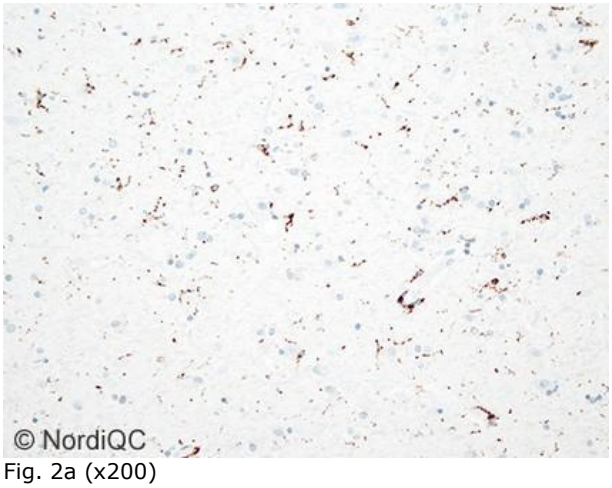


Fig. 2a (x200)
Optimal staining reaction for CD68 of the brain using the same protocol as in Fig. 1a. The microglial cells show a weak to moderate distinct cytoplasmic staining reaction - compare with Fig. 2b.
Brain was found to be superior to tonsil to evaluate the low limit of CD68 demonstration.

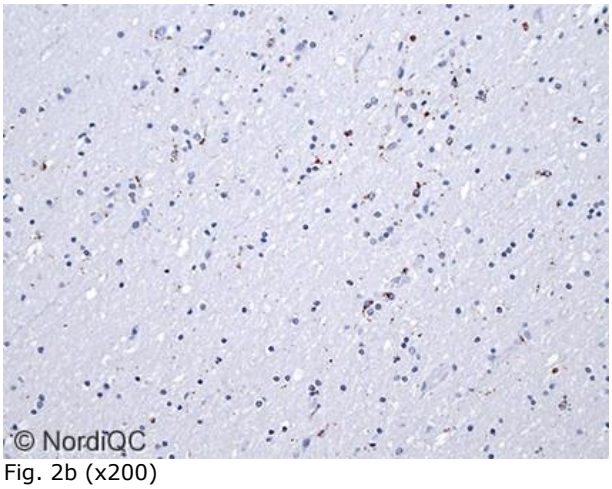


Fig. 2b (x200)
Insufficient staining reaction for CD68 of the brain using the same protocol as in Fig. 1b. The proportion and intensity of microglial cells demonstrated being significantly reduced - compare with Fig. 2a.

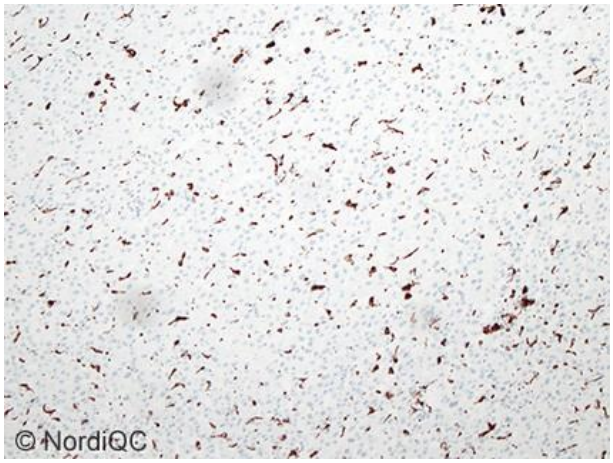


Fig. 3a (x100)
Optimal staining reaction for CD68 of the liver using the same protocol as in Figs. 1a and 2a. The Kupffer cells show a moderate to strong cytoplasmic staining reaction, while the hepatocytes are negative - compare with Fig. 3b.

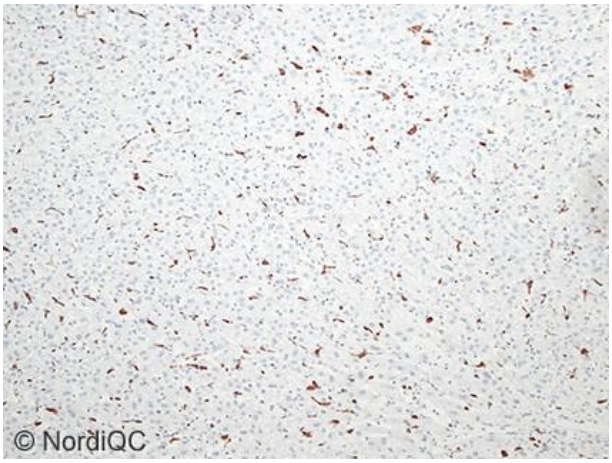


Fig. 3b (x100)
Staining reaction of the liver using the same protocol as in Figs. 1b and 2b. The Kupffer cells show a weak to strong cytoplasmic staining reaction, while the hepatocytes are negative - compare with Fig. 3a.
Also compare with Figs. 4b and 5b.

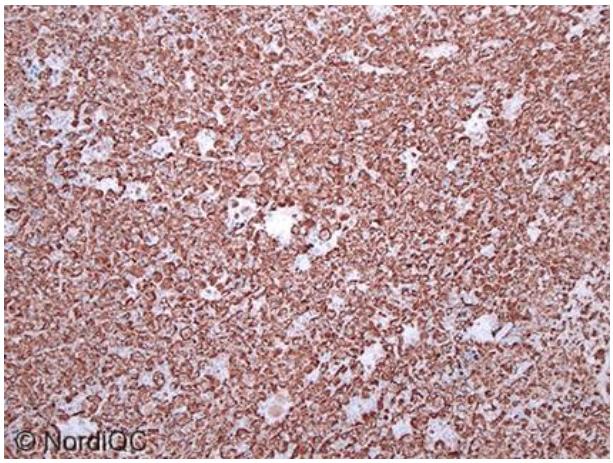


Fig. 4a (x100)
Optimal staining reaction for CD68 of the histiocytic sarcoma using the same protocol as in Figs. 1a – 3a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic staining reaction - compare with Fig. 4b.

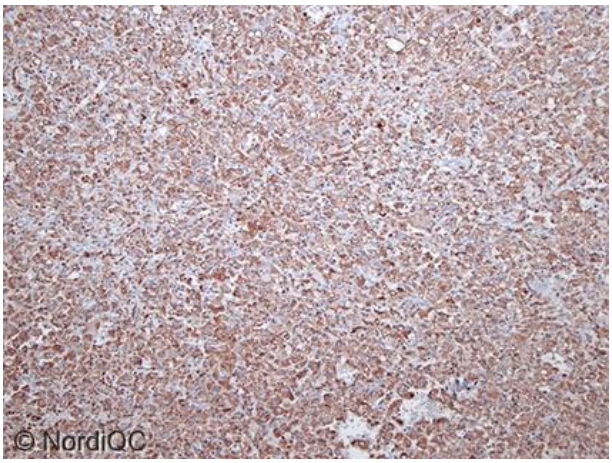


Fig. 4b (x100)
Staining reaction for CD68 of the histiocytic sarcoma using the same protocol as in Figs. 1b – 3b.
Virtually all the neoplastic cells show a moderate to strong cytoplasmic staining reaction - compare with Fig. 4a.

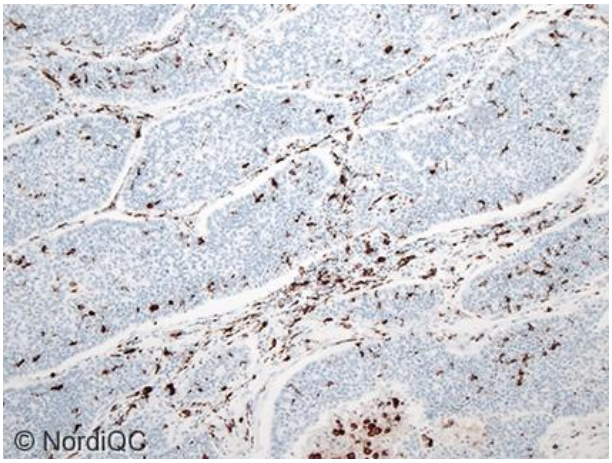


Fig. 5a (x100)
Optimal staining reaction for CD68 of the lung adenocarcinoma using the same protocol as in Figs. 1a. – 4b. All the neoplastic cells are negative while the macrophages show a moderate to strong cytoplasmic staining reaction - compare with Fig. 5b.

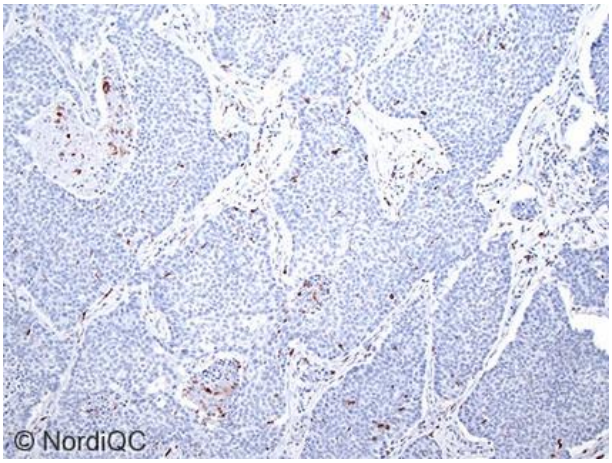


Fig. 5b (x100)
Insufficient staining reaction for CD68 of the lung adenocarcinoma using the same protocol as in Figs. 1b – 4b. The neoplastic cells are negative, but the number of macrophages demonstrared is strongly reduced - compare with Fig. 5a.

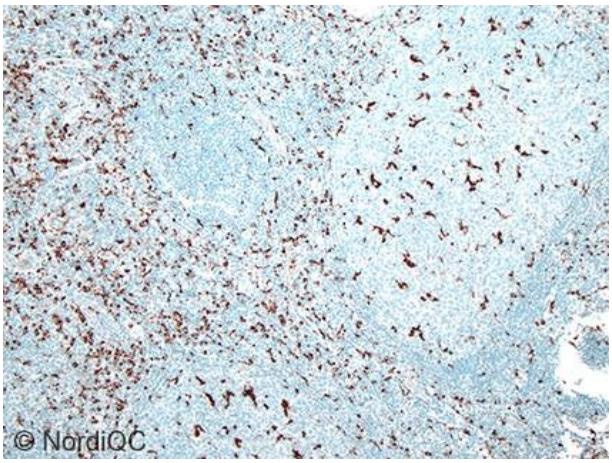


Fig. 6a (x100)
Optimal staining reaction for CD68 of the tonsil using the mAb clone KP1 as RTU (790-2931) 12 min., HIER in an alkaline buffer (CC1) for 40 min. and a multimer-based detection system (OptiView with amplification kit, Ventana) - same protocol used in Fig. 7a. The germinal center and interfollicular macrophages show a moderate to strong distinct cytoplasmic staining reaction, and no background or staining of the germinal centre B-cells is seen - compare with Fig. 6b.

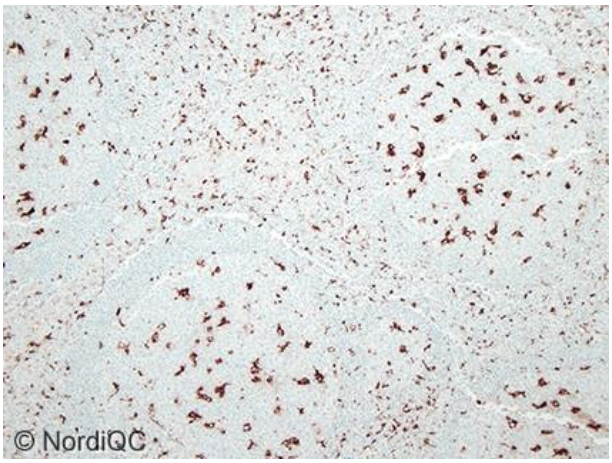


Fig. 6b (x100)
Staining reaction for CD68 of the tonsil using the mAb clone KP1 as RTU (790-2931) 24 min., HIER in an alkaline buffer (CC1) for 16 min. and a multimer-based detection system (OptiView, Ventana), - same protocol used in Fig. 7b. The intensity of the staining reaction, and the amount of the interfollicular macrophages is reduced compared to level seen by optimal protocol settings - compare with Fig. 6a.
However, also see Fig. 7b, same protocol.

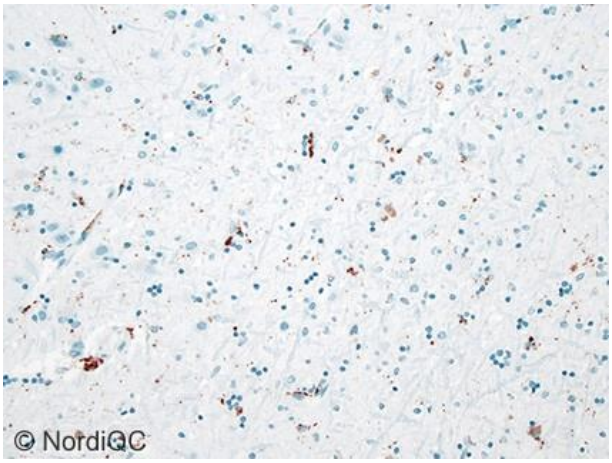


Fig. 7a (x200)
Optimal staining reaction for CD68 of the brain using the same protocol as in Fig. 6a. The microglial cells show a weak to moderate distinct cytoplasmic staining reaction - compare with Fig. 7b.
Brain was found to be superior to tonsil to evaluate the low limit of CD68 demonstration.

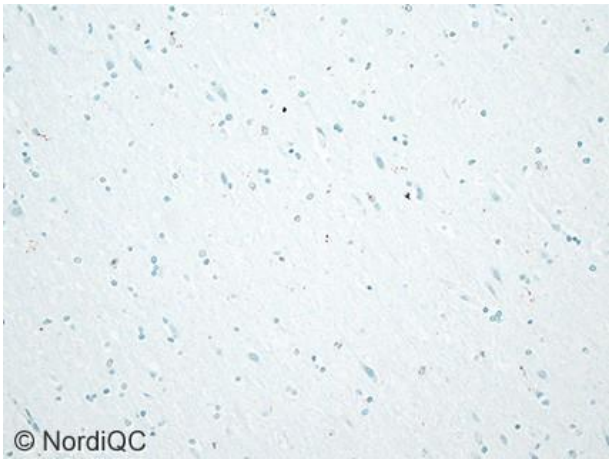


Fig. 7b (x200)
Insufficient staining reaction for CD68 of the brain using the same protocol as in Fig. 6b. The microglial cells are virtually all false negative - compare with Fig. 7a.

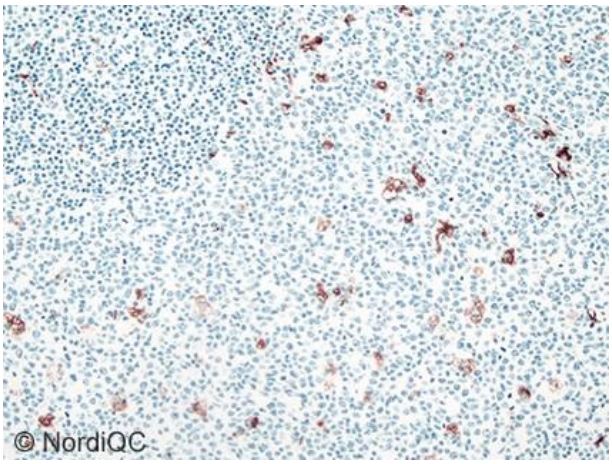


Fig. 8a (x200)
Insufficient staining reaction for CD68 of the tonsil using the mAb clone PG-M1 as conc 1:60 with proteolytic pretreatment (Protease 1, Ventana) and IVIEW (Ventana) as detection system - same protocol used in Fig. 8b. Only germinal center macrophages show a moderate to strong cytoplasmic staining reaction but in general the morphology is impaired due to excessive proteolysis and digestion of the fragile membranes of especially the interfollicular macrophages reducing the number of cells demonstrated.

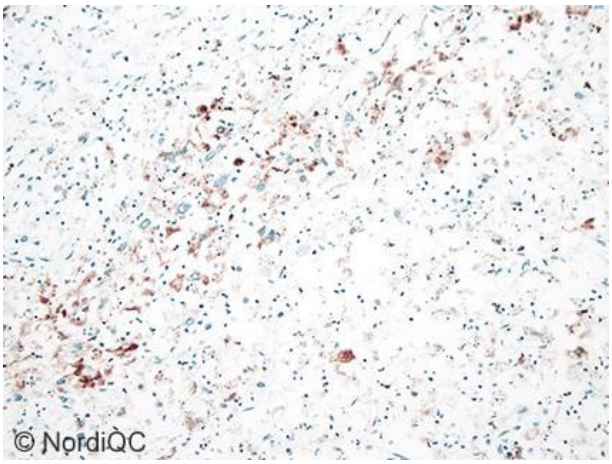


Fig. 8b (x200)
Insufficient staining of CD68 of the histiocytic sarcoma same protocol as in Fig. 8a. The morphology is strongly impaired due to the proteolytic pretreatment and complicates the interpretation.

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