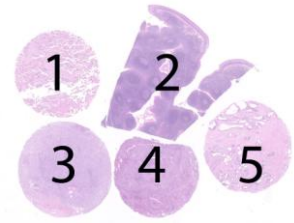
Assessment Run 44 2015
p40 (ΔNp63)

Material
The slide to be stained for p40 comprised:



1. Placenta, 2. Tonsil, 3. Lung adenocarcinoma, 4. Lung squamous cell carcinoma, 5. Prostate hyperplasia

All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing p40 staining as optimal included:

- A moderate to strong, distinct nuclear staining reaction of virtually all squamous epithelial cells in the tonsil and basal cells lining the hyperplastic glands in the prostate
- An at least weak to moderate, distinct nuclear staining reaction of dispersed cytotrophoblastic cells in the placenta
- A moderate to strong, distinct nuclear staining reaction of the vast majority of neoplastic cells in the lung squamous cell carcinoma
- No staining reaction of neoplastic cells in the lung adenocarcinoma
- No staining reaction of other cells including lymphocytes in the tonsil

Participation

Number of laboratories registered for p40, run 44	141
Number of laboratories returning slides	130 (92%)

Results
130 laboratories participated in this assessment, one submitted a slide using an inappropriate antibody. Of the remaining 129 laboratories, 56% achieved a sufficient mark (optimal or good). Table 1 summarizes antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:
- Use of less successful primary antibodies
- Too low concentration of the primary antibody
- Use of less sensitive detection systems

Performance history
This was the first NordiQC assessment of p40. A relatively low pass rate of 56% was observed.

Table 2. **Proportion of sufficient results for p40 in the 1'st NordiQC run performed**

	Run 44 2015
Participants, n=	129
Sufficient results	56%

Conclusion
The mAb clones **BC28** and **ZR8** were the most successful antibodies and could both be used to obtain an optimal staining result for p40. The concentrated formats of the most commonly used mAb clone **BC28** provided optimal staining result on the three main platforms from Dako, Leica and Ventana.
Irrespective of the clone applied, efficient HIER and use of a sensitive and specific 3-step polymer / multimer based detection system gave the highest proportion of optimal results. The concentration of the primary antibody must be carefully calibrated. All polyclonal antibodies applied in this assessment gave less successful results.
Placenta is recommended as positive tissue control for p40 where an at least a weak to moderate, distinct nuclear staining reaction of dispersed cytotrophoblasts must be seen.
Tonsil can be used as negative tissue control. No nuclear staining reaction in lymphocytes should be seen.

Table 1. **Antibodies and assessment marks for p40, run 44**

Concentrated antibodies	n	Vendor	Optimal	Good	Borderline	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **BC28**	49 3 1	Biocare Zytomed Nordic Biosite	26	17	10	0	81%	86%
rmAb clone **ZR8**	12 1	Immunologic Zeta Corporation	3	6	4	0	69%	80%
pAb **AC13030**	16	Biocare	0	3	11	2	19%	-
pAb **PC373**	9	Calbiochem, Merck	0	2	7	0	22%	-
pAb **RP163**	5 1 1	Diagnostic Biosystems Medac diagnostica ITK DIAGNOSTICS BV	0	2	4	1	29%	-
pAb **ab99513***	4	Abcam	0	0	4	0	-	-
pAb **PDR 055**	1 1	Diagnostic Biosystems ITK DIAGNOSTICS BV	0	1	0	1	-	-
pAb **RBK054**	2	Zytomed	0	1	1	0	-	-
pAb **ab166857**	1	Abcam	0	0	0	1	-	-
pAb **ab167612**	1	Abcam	0	0	0	1	-	-
pAb **BP4206**	1	ID Labs	0	1	0	0	-	-
pAb **ILP-3726**	1	Immunologic	0	0	1	0	-	-
pAb **Unknown**	1	Unknown	0	0	1	0	-	-
Ready-To-Use antibodies								
mAb clone **BC28 API 3066**	6	Biocare	3	2	1	0	83%	100%
mAb clone **BC28 790-4950**	2	Ventana	1	1	0	0	-	-
pAb **API 3030**	3	Biocare	0	2	1	0	-	-
pAb **RAB-066**	3	Maixin	0	1	2	0	-	-
pAb **PDR 055**	2	ITK DIAGNOSTICS BV	0	0	2	0	-	-
mAb **MAD-000623QD**	2	Master Diagnostica	0	0	2	0	-	-
Total	129		33	39	51	6	-	
Proportion			26%	30%	40%	4%	56%	

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.

Detailed analysis of p40, Run 44
The following protocol parameters were central to obtain optimal staining:

Concentrated antibodies
mAb clone **BC28**: Protocols with optimal results were all based on HIER using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (3/8)*, TRS pH 9 (Dako) (4/7), TRS pH 6.1 (Dako) (1/1), Cell Conditioning 1 (CC1, Ventana) (15/25), Bond Epitope Retrieval Solution 2 (BERS2, Leica) (1/4) or Citrate pH 6 (2/3) as retrieval buffer. The mAb was typically diluted in the range of 1:25-1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 37 of 43 (86%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

rmAb clone **ZR8**: Protocols with optimal results were all based on HIER using TRS pH 9 (Dako) (1/1), CC1 (Ventana) (1/3) or Tris-EDTA pH 9 (1/1) as retrieval buffer. The rmAb was diluted in the range of 1:100-1:800 depending on the total sensitivity of the protocol employed. Using these protocol settings 4 of 5 (80%) laboratories produced a sufficient staining result.

Table 3. **Proportion of optimal results for p40 for the most commonly used antibody as concentrate on the 3 main IHC systems***

Concentrated antibodies	**Dako Autostainer Link / Classic**		**Ventana BenchMark XT / Ultra**		**Leica Bond III / Max**	
	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 clone **BC28**	7/15** (47%)	-	13/18 (72%)	-	1/3	-

* 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 **BC28** product no. **API 3066**, Biocare, intelliPATH:
One protocol with an optimal result was based on HIER using Diva Decloaker pH 6.2 in a Pressure Cooker (efficient heating time 15 min. at 110°C), 30 min. incubation of the primary Ab and MACH4 Universal HRP-Polymer (M4U534) as detection system.

mAb clone **BC28**, product no. **790-4950**, Ventana, BenchMark ULTRA:
One protocol with an optimal result was based on HIER using Cell Conditioning 1 (efficient heating time 32 min.), 16 min. incubation of the primary Ab and OptiView (760-700) as detection system.

Comments
In this first NordiQC assessment for p40 the prevalent feature of an insufficient result was a too weak or completely false negative staining reaction of the cells expected to be demonstrated. This pattern was seen in 57% of the insufficient results (29 of 51 laboratories). The remaining insufficient results were characterized by a general poor signal-to-noise ratio, excessive background reaction and/or aberrant cytoplasmic staining reaction in e.g. cytotrophoblasts and lymphocytes complicating the interpretation. Too weak staining was typically characterized by a reduced staining reaction both in regard to the intensity and proportion of cells expected to be demonstrated. This was in particular observed in the cytotrophoblasts of placenta and basal cells of prostate glands, whereas virtually all laboratories successfully demonstrated p40 in the majority of neoplastic cells of the lung squamous cell carcinoma. A too weak staining reaction was most frequently caused by a too low titre of an otherwise well performing primary antibody as mAb clone BC28. Poor signal-to-noise ratio typically was caused by a less successful primary antibody.

The mAb clone BC28 gave the highest proportion of sufficient and optimal results, as seen in table 1. BC28 was the most frequently used concentrated format within a laboratory developed (LD) assay and optimal results could be obtained on the 3 most widely used IHC platforms, as shown in table 3. Efficient HIER in an alkaline buffer in combination with a 3-step polymer/multimer based detection system provided the highest proportion of optimal results. E.g. on the Ventana BenchMark platform 9 of 11 protocols (82%) provided an optimal result if mAb clone BC28 was used as a concentrate in the range of 1:25-500, HIER was performed in CC1 for 32-64 min. and OptiView (760-700) as detection system. Using similar settings but applying UltraView (760-500) as detection system only 5 of 13 protocols (38%) provided optimal results.

11 different polyclonal Abs (pAb) were used as concentrates by the laboratories within LD assays (45 protocols in total). Despite protocol settings, as retrieval conditions, detection systems and IHC stainer platforms, were identical to the mAb clones BC28 and ZR8, no optimal result was provided and the overall pass rate for laboratories using a pAb within a LD assay was 22% (10 of 45).
The insufficient results were typically characterized by a poor signal-to-noise and aberrant staining reaction compromising the interpretation. The most commonly used pAb AC13030 (BioCare) gave a cytoplasmic staining reaction in e.g. cytotrophoblasts and smooth muscle cells, whereas the pAbs RP163 (various vendors) and ab99513 (Abcam) cross reacted with B-cells. From the protocols submitted it seemed impossible to eliminate the aberrant staining reactions e.g. by reducing the titre of the primary Ab as this impaired the required sensitivity especially in p40 low-level expressing structures.

Corresponding Ready-To-Use (RTU) formats provided the same results as seen for concentrated formats, though the numbers of protocols were limited and conclusions must be drawn with caution.
Optimal results were obtained by the RTU system from Biocare based on the mAb clone BC28, API3066 using the official protocol recommendation, which in brief was based on HIER in Diva buffer, MACH4 as detection system and intelliPATH™ as IHC stainer.
mAb clone BC28 was also successfully applied as RTU system from Ventana. The optimal result was based on the RTU format 790-4950, HIER in CC1 Mild and OptiView as detection system.

Controls
Placenta is recommended as positive tissue control for p40, where an at least weak to moderate, distinct nuclear staining reaction of cytotrophoblasts must be seen.
Supportive to placenta, tonsil can be used as positive and negative tissue control. Virtually all squamous epithelial cells must show a moderate to strong, distinct nuclear staining reaction. No nuclear or cytoplasmic staining reaction should be seen in other cell types.

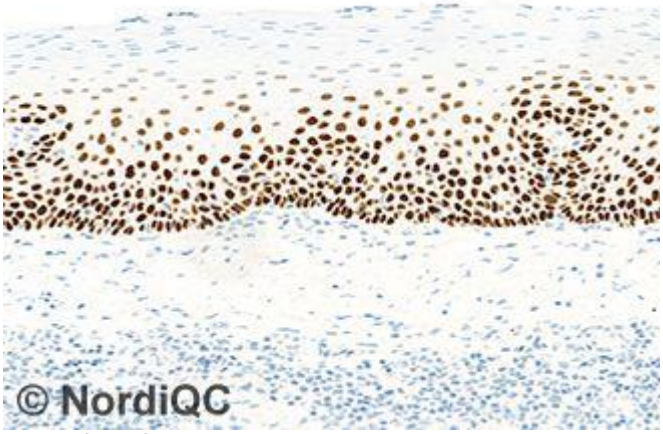


Fig. 1a (x200)
Optimal p40 staining of the tonsil using the mAb clone BC28 as a concentrate, optimally calibrated, HIER in an alkaline buffer (TRS pH 9.0, Dako), and a 3-step polymer based detection system (FLEX+, Dako). A moderate to strong nuclear staining reaction is seen in the majority of the squamous epithelial cells. No background staining is seen. Same protocol used in Figs. 1a - 4a.

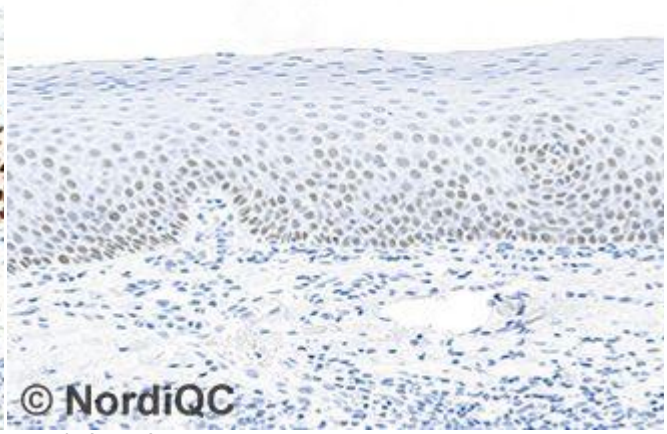


Fig. 1b (x200)
Insufficient p40 staining of the tonsil using the mAb clone BC28. The protocol provided an overall too low sensitivity most likely due to a combination of a too low concentration of the primary Ab and use of a 2-step polymer based detection system with a moderate sensitivity (FLEX, Dako)- compare with Fig. 1a (same field). The intensity and proportion of cells demonstrated is reduced. Also compare with Figs. 2b - 4b, same protocol.

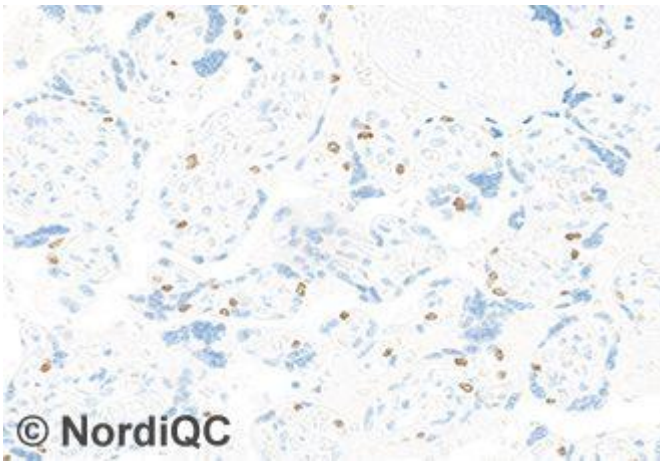


Fig. 2a (x200)
Optimal p40 staining of the placenta using same protocol as in Fig. 1a. Scattered cytothrophoblastic cells show a weak to moderate, distinct nuclear staining reaction.

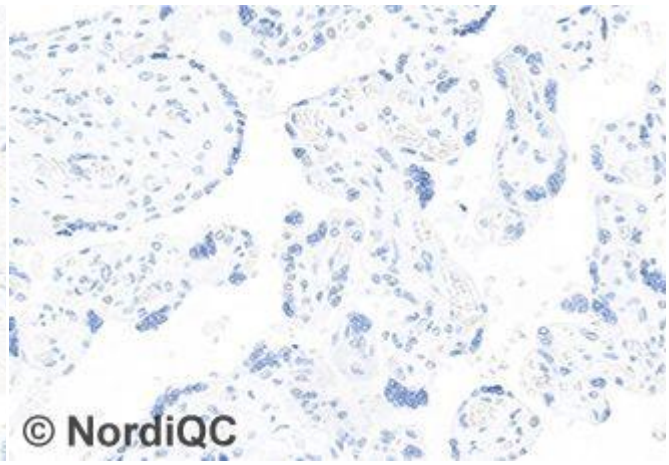


Fig. 2b (x200)
Insufficient p40 staining of the placenta using same protocol as in Fig. 1b. Virtually no staining reaction of cytothrophoblastsic cells is seen. Also compare with Figs. 3b and 4b, same protocol.

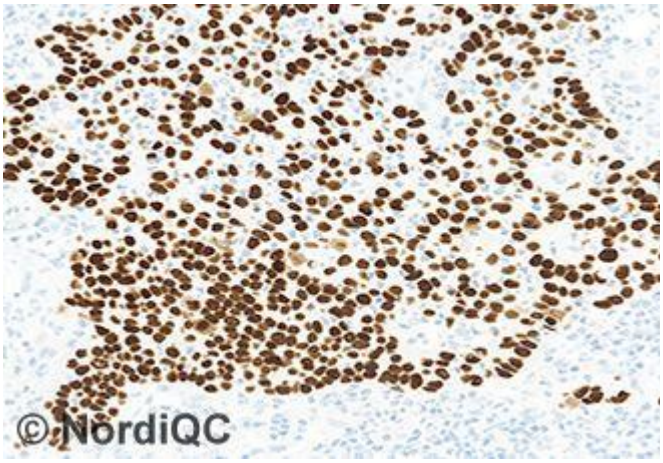


Fig. 3a (x200)
Optimal p40 staining of the lung squamous cell carcinoma using same protocol as in Figs. 1a & 2a.Virtually all neoplastic cells show a moderate to strong nuclear staining reaction. No background staining is seen.

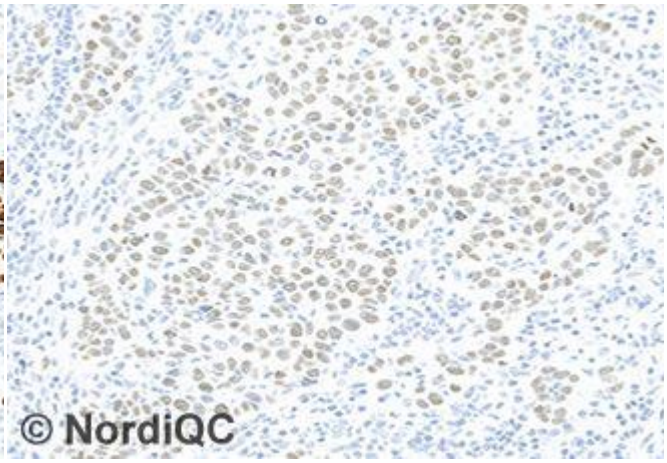


Fig. 3b (x200)
Insufficient p40 staining of the lung squamous cell carcinoma using same protocol as in Figs. 1b & 2b. The intensity and proportion of cells demonstrated is significantly reduced.

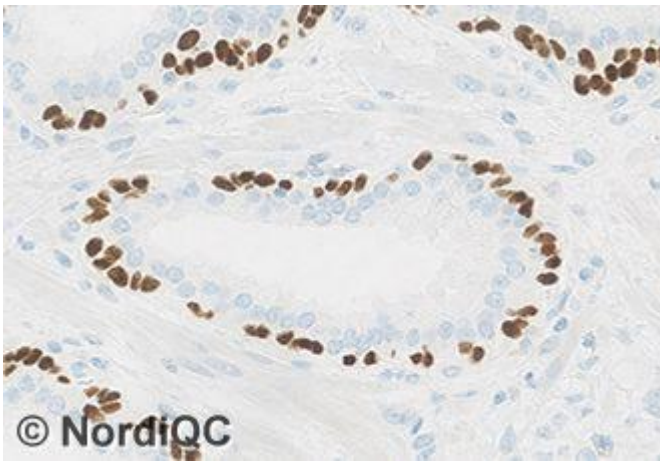


Fig. 4a (x400)
Optimal p40 staining of the prostate hyperplasia using same protocol as in Figs. 1a - 3a. The basal cells are distinctively demonstrated as a moderate to strong nuclear staining reaction is observed.

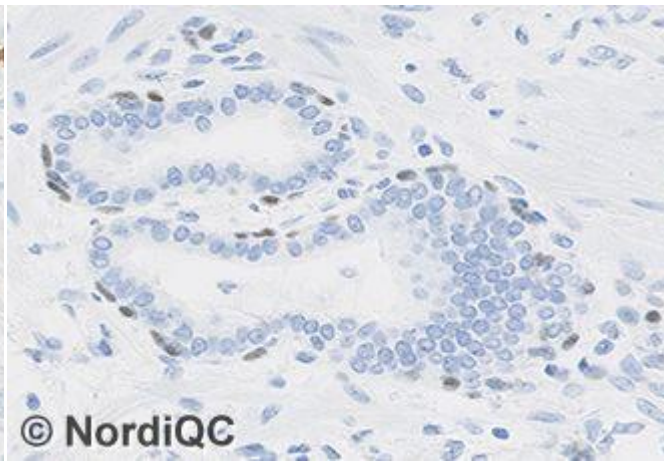


Fig. 4b (x400)
Insufficient p40 staining of the prostate hyperplasia using same protocol as in Figs. 1b – 3b. Only a weak and equivocal nuclear staining reaction in the basal cells is observed.

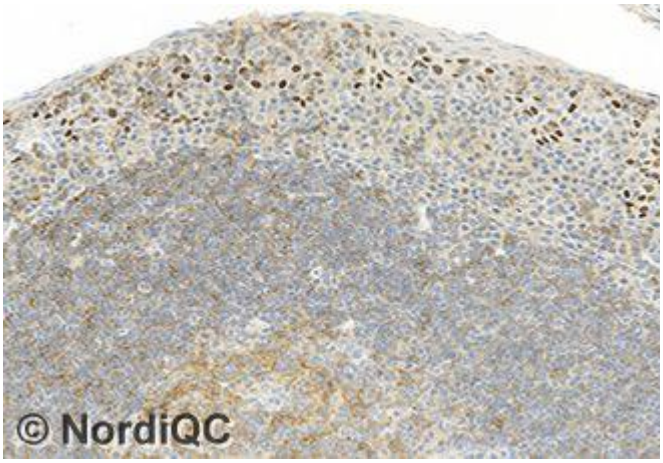


Fig. 5a (x100)
Insufficient p40 staining of the tonsil using a pAb (ab99513, Abcam). The interpretation is compromised primarily due to a poor signal-to-noise ratio and a general background reaction but also to an aberrant staining reaction of B-cells. This pattern was seen for pAbs ab99513 and RP163.

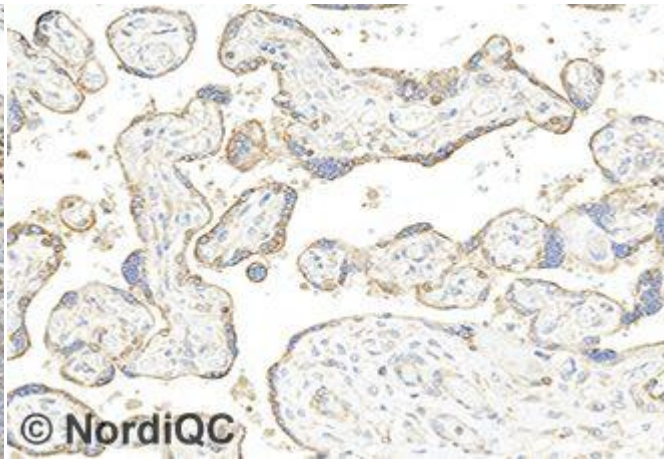


Fig. 4b (x100)
Insufficient p40 staining of the placenta using a pAb (AC13030, Biocare). An excessive cytoplasmic staining reaction compromises the interpretation in especially low-level p40 expressing cells as cytotrophoblasts. This pattern was frequently seen for pAbs AC13030 and PC373.

SN/RR/LE/MV 24-6-2015