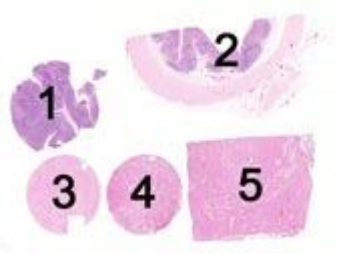
Assessment Run 36 2012
Hepatocyte antigen (Hepa)

Material

The slide to be stained for Hepa (HepPar1) comprised:

1. Tonsil, 2. Appendix, 3. Liver, 4. & 5. Hepatocellular carcinoma



All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a Hepa staining as optimal included:

- A moderate to strong granular cytoplasmic staining reaction of virtually all the hepatocytes in the liver and no staining reaction of the bile ductal epithelial cells.
- An at least weak to moderate and distinct granular cytoplasmic staining reaction of the majority of the neoplastic cells in the two hepatocellular carcinomas.
- No staining reaction in the vast majority of the columnar epithelial cells of the appendix. A moderate to strong cytoplasmic staining reaction focally in the columnar epithelial cells could be seen.
- No staining reaction in the tonsil.

158 laboratories participated in this assessment. 86 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for Hepa, run 36**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **OCH1E5**	93 6 5 2 1 1	Dako Leica/Novocastra Thermo/NeoMarkers Cell Marque Diagnostic Biosystems Maxim	48	38	19	3	80 %	90 %
Ready-To-Use Abs								
mAb clone **OCH1E5 IS/IR624**	25	Dako	21	4	0	0	100 %	100 %
mAb clone **OCH1E5 760-4350**	20	Ventana/Cell Marque	18	2	0	0	100 %	100 %
mAb clone **OCH1E5 264M-97/98**	3	Cell Marque	2	1	0	0	-	-
mAb clone **OCH1E5 BSB 5629**	1	BioSB	1	0	0	0	-	-
mAb clone **OCH1E5 113-03**	1	Master Diagnostica	0	1	0	0	-	-
Total	158		90	46	19	3	-	
Proportion			57 %	29 %	12 %	2 %	86 %	

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

Detailed analysis of Hepa, Run 36

Following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs

mAb clone **OCH1E5**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER) using either Target Retrieval Solution pH 9 (3-in-1) (TRS pH 9; Dako) (14/20)*, TRS pH 9 (Dako) (4/7), Cell Conditioning 1 (CC1; Ventana) (24/42), Tris-EDTA/EGTA pH 9 (4/13) or Citrate pH 6 (2/4) as the retrieval buffer.
The mAb was typically diluted in the range of 1:30-1:1.200 depending on the total sensitivity of the protocol

employed. Using these protocol settings 75 out of 83 (79 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this reagent)

Ready-To-Use Abs
mAb clone **OCH1E5** (prod. no. IS/IR624, Dako): The protocols giving an optimal result were typically based on HIER in PT-Link (heating time for 10-20 min at 95-98°C) using TRS pH 9 (3-in-1) (Dako) or TRS pH 9 (Dako) as HIER buffer, an incubation time of 20-30 min in the primary Ab and EnVision Flex or EnVision Flex+ (K8000/K8002) as the detection system. Using these protocol settings 24 out of 24 (100 %) laboratories produced a sufficient staining.

mAb clone **OCH1E5** (prod. no. 760-4360, Ventana/Cell Marque): The protocols giving an optimal result were typically based on HIER using mild or standard CC1, an incubation time of 16-40 min in the primary Ab UltraView (760-500) or OptiView (760-700) as the detection system. Using these protocol settings 20 out of 20 (100 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient staining results were:
-Less successful performance of the mAb clone OCH1E5 on the Bond (Leica) platform
-Too low concentration of the primary antibody

In this first NordiQC assessment of Hepa, virtually all laboratories were able to demonstrate Hepa in the normal liver cells, whereas the prevalent feature of the insufficient results was an aberrant and most likely false positive staining reaction in cells not expected to be demonstrated. The aberrant staining reaction was particulary observed in the germinal centre B-cells and in interfollicular macrophages, but also seen in e.g. plasma cells and squamous epithelial cells of the tonsil. For unexplained reasons this staining pattern was mainly observed, when the mAb clone OCH1E5 was used on the Bond IHC platform, Leica using HIER in either ER 1 or ER 2 and a 3-step polymer based detection system, Refine. The primary mAb was used in the range of 1:50-3.000. In total 12 out 17 protocols performed on the Bond IHC platform gave an extensive aberrant staining reaction marked as insufficient. None of the remaining 5 protocols were assessed as optimal.
It was difficult to identify the reason(s) for the aberrant staining pattern on the Leica IHC platform, as the same mAb clone OCH1E5 applied with similar protocol settings on other IHC platforms such as the Dako Autostainer system, the Ventana BenchMark ULTRA and XT, gave optimal staining reactions. Both the range of the titre of the primary Ab, HIER settings and application of sensitive 3-step polymer or multimer based detection systems were comparable. Different washing buffers incl chemical compositions and/or washing procedures used on the Leica Bond platform compared to the other IHC platforms can be a cofactor.
NordiQC is in contact with Leica in order to elucidate the aberrant staining pattern. As no data or literature indicates that Hepa should be expressed in germinal centre B-cells etc, the protocols giving a distinct staining reaction in these cells are considered insufficient.
In the remaining insufficient results, a too weak staining reaction was seen and characterized by a reduced staining in both the normal hepatocytes and in the neoplastic cells of the two hepatocellular carcinomas. This staining pattern was typically caused by a too low concentration of the primary Ab.

The two most commonly used Ready-To-Use systems for Hepa from Dako and Ventana both provided a pass rate of 100 % thus being superior to the in-house optimized assays.

Liver was found to be a recommendable positive control for Hepa: Virtually all liver cells must show an at least moderate and distinct granular cytoplasmic staining reaction, while no staining should be seen in the background or in the the bile ducts.
It has to be emphasized that the use of biotin based detection systems cannot be recommended, as it is impossible to differentiate the specific granular staining reaction of Hepa from an unspecific staining reaction due to endogenous biotin as both Hepa and biotin are localized in the liver cell mitochondria.

Conclusion
The mAb clone **OCH1E5** both as a concentrate and as a Ready-To-Use system from Dako and Ventana is highly recommended as marker for Hepa. HIER and usage of a non-biotin based detection system is mandatory for an optimal performance.
Liver can be used as positive control, in which virtually all liver cells must show an at least moderate granular cytoplasmic staining reaction. Tonsil can be used as negative control – no staining should be seen.

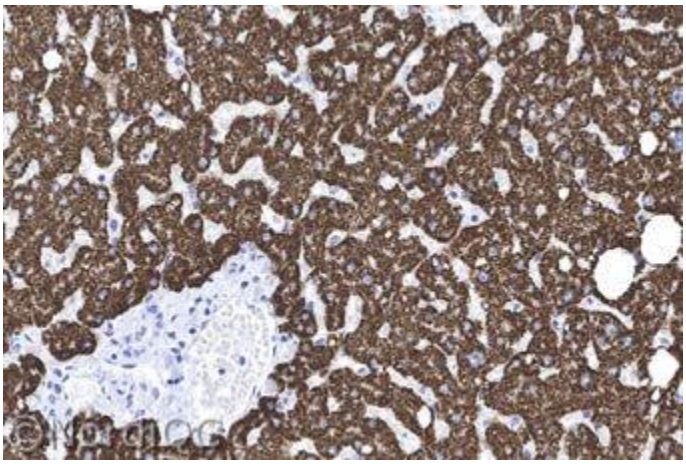


Fig. 1a
Optimal Hepa staining of the liver using the mAb clone OCH1E5 optimally calibrated and with HIER. Virtually all the hepatocytes show a strong, granular cytoplasmic staining reaction. No background staining or staining of the bile ductal epithelial cells is seen.

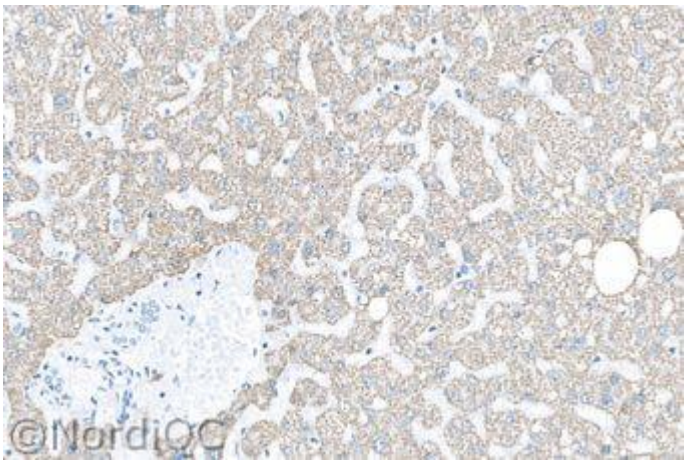


Fig. 1b
Insufficient staining for Hepa of the liver, using the mAb clone OCH1E5 with protocol settings giving a too low sensitivity (too low concentration of the primary Ab) - same field as in Fig. 1a. The intensity of the cells demonstrated is significantly reduced. Also compare with Fig. 2b – same protocol.

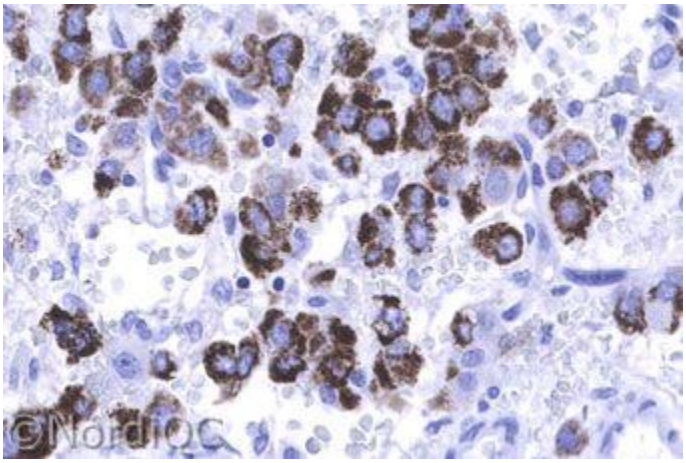


Fig. 2a
Optimal Hepa staining of the hepatocellular carcinoma, tissue no. 4 in the NordiQC multiblock using same protocol as in Fig. 1a.
Virtually all the neoplastic cells show a strong and distinct cytoplasmic staining reaction. No background staining is seen.

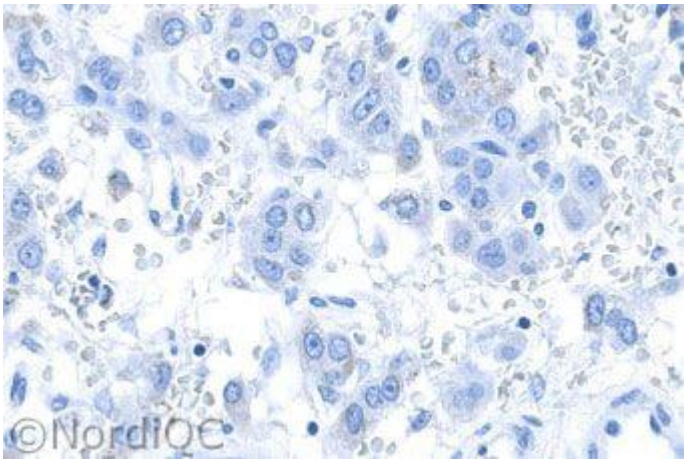


Fig. 2b
Insufficient staining for Hepa of the hepatocellular carcinoma, tissue no. 4 in the NordiQC multiblock using same protocol as in Fig. 1b – same field as in Fig. 2a.
Only scattered neoplastic cells show a weak and equivocal staining reaction.

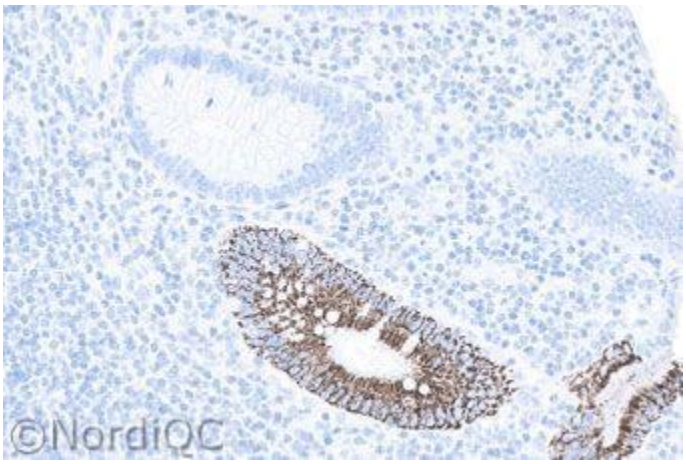


Fig. 3a
Optimal Hepa staining of the appendix using the mAb clone OCH1E5 optimally calibrated, HIER in TRS pH 9 (Dako), using EnVision FLEX as the detection system and applied on the Dako Autostainer system.
In some crypts the columnar epithelial cells show a strong granular cytoplasmic staining reaction. No background staining or staining of the stromal cells is seen.

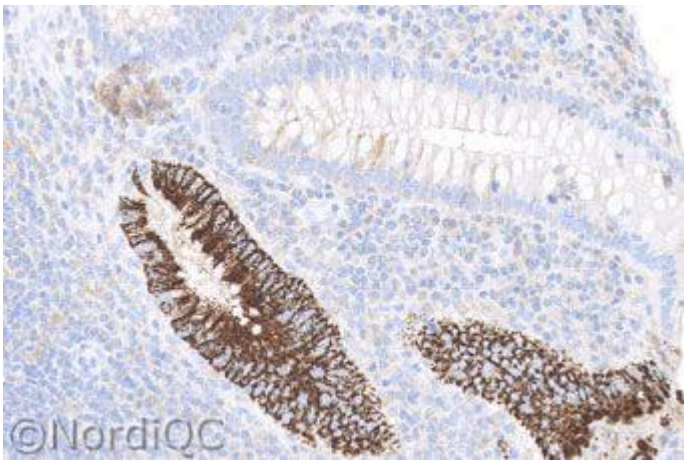


Fig. 3b
Insufficient Hepa staining of the appendix using the mAb clone OCH1E5, HIER in ER 2 (Leica) and Bond Refine as the detection system.
In addition to the focal staining reaction in the columnar epithelial cells a weak background staining and staining in the stromal cells is seen.
Also compare with Fig. 4b - same protocol.

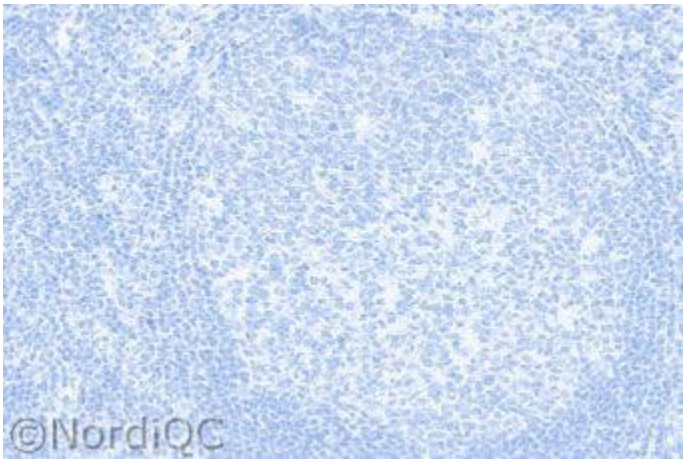


Fig. 4
Optimal Hepa staining of the tonsil using same protocol as in Fig. 3a. No staining reaction is seen.

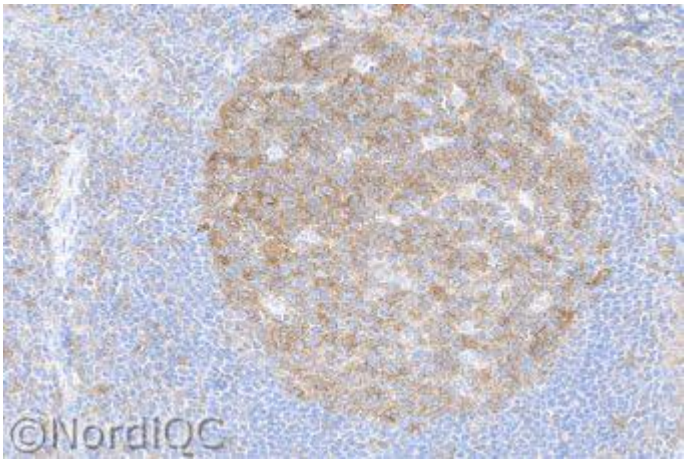


Fig. 4b
Insufficient Hepa staining of the tonsil using same protocol as in Fig. 3b.
The germinal centre B-cells show a moderate intracytoplasmic staining reaction and a general weak background staining is seen.
This aberrant staining pattern was typically seen, when the mAb clone OCH1E5 was applied on the Bond IHC platform, Leica.

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