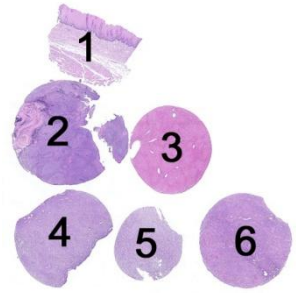
Assessment Run 28 2010
CD99



The slide to be stained for CD99 comprised:
1. Esophagus, 2. Tonsil, 3. Liver, 4-5. peripheral primitive neuroectodermal tumour (pPNET - Ewing sarcoma), 6. Granulosa cell tumour.
All tissues were fixed in 10% neutral buffered formalin.

Criteria for assessing a CD99 staining as optimal included:

- A moderate to strong distinct predominantly membranous staining of the basal and parabasal squamous epithelial cells in the esophagus and tonsil.
- A moderate to strong predominantly membranous staining in lymphocytes and endothelial cells in all the specimens.
- An at least weak to moderate predominantly membranous staining of the majority of the neoplastic cells of the two pPNETs and the granulosa cell tumour.
- No cytoplasmic staining in the liver cells, the intermediate and superficial squamous epithelial cells.

106 laboratories participated in this assessment. 1 laboratory used an inappropriate Ab. Of the remaining 105 laboratories 34 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CD99, run 28**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb clone **12E7**	55	Dako	4	16	13	22	36 %	62 %
mAb clone **013**	5 3 1 1	NeoMarkers Covance Master Diagnostica Zymed	0	3	1	6	30 %	-
mAb clone **H036-1.1**	5 2 2	Novocastra Biocare NeoMarkers	0	0	0	9	0 %	-
rmAb clone **EPR3097Y**	5 1	Epitomics Cell Marque	3	3	0	0	100 %	100 %
Ready-To-Use Abs								
mAb clone **12E7, IR057**	11	Dako	2	4	4	1	55 %	86 %
mAb clone **12E7, N1593**	1	Dako	0	1	0	0	-	-
mAb clone **12E7, PA0509**	2	Leica	0	0	0	2	-	-
mAb clone **H036-1.1, 760-2631**	9	Ventana/Cell Marque	0	0	0	9	0 %	-
mAb clone **H036-1.1, PM008**	1	Biocare	0	0	0	1	-	-
mAb clone **013, 790-4452**	1	Ventana	0	0	0	1	-	-
Total	105		9	27	18	51	-	-
Proportion			8 %	26 %	17 %	49 %	34 %	-

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

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

Concentrated Abs
mAb clone **12E7**: The protocols giving an optimal result were all based on heat induced epitope retrieval (HIER)

using either Tris-EDTA/EGTA pH 9 (1/8)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako)(1/12), Cell Conditioning 1 (BenchMark, Ventana) (1/11) or EDTA/EGTA pH8 (1/2) as the retrieval buffer. The mAb was typically diluted in the range of 1:50– 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 13 out of 21 (62 %) laboratories produced a sufficient staining (optimal or good).
* (number of optimal results/number of laboratories using this buffer)

rmAb clone **EPR3097Y**: The protocols giving an optimal result were all based on HIER using either Tris-EDTA/EGTA pH 9 (1/2) or Cell Conditioning 1 (BenchMark, Ventana) (2/2) as the retrieval buffer. The mAb was typically diluted in the range of 1:1.000– 1:2.000 depending on the total sensitivity of the protocol employed. Using these protocol settings all of 4 laboratories produced a sufficient staining.
* (number of optimal results/number of laboratories using this buffer)

Ready-To-Use Abs
mAb clone **12E7** (prod. no. IR057 Dako): The protocols giving an optimal result were both based on HIER for 20 min. in PT-Link using Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH), an incubation time of 20 min in the primary Ab and EnVision Flex (K8000) or Flex+ (K8002) as the detection system. Using these protocol settings 6 out of 7 (86 %) laboratories produced a sufficient staining.

The most frequent causes of insufficient staining results were:
- Less successful primary Ab
- Too low concentration of the primary Ab
- Insufficient HIER – typically usage of citrate pH 6
- Omission of epitope retrieval.

In this assessment and in concordance with the assessment of CD99 in run 12, 2004, the overall feature of an insufficient staining was a too weak or completely false negative staining reaction of the cells expected to be demonstrated. This pattern was seen in virtually all the 69 stains assessed as insufficient. As also observed in run 12, tonsil or esophagus should be preferred as a positive control for CD99 provided that a moderate to strong, distinct membranous reaction is seen in the basal squamous epithelial cells. Virtually all laboratories obtaining this reaction pattern were also capable to demonstrate CD99 sufficiently in the two pPNETS and the granulosa cell tumour.
In this assessment the newly launched Ab for CD99, rmAb EPR3097Y gave a significantly higher pass rate than the old mAb clones for CD99 (particularly clone H036-1.1, which showed a poor performance - see Table 1).
In many cases mAb clone 12E7 gave a mainly dot-like cytoplasmic staining in the pPNETs, while in other cases a more membranous staining was seen. From the protocols submitted, it was not possible to identify any explanation for the different reaction patterns. Both the membranous and the dot-like reaction were accepted.
This was the second assessment of CD99 in NordiQC. The proportion of sufficient results in both runs has been very low – see table 2.

Table 2. **Proportion of sufficient results for CD99 in the two NordiQC runs performed**

	Run 12 2004	Run 28 2010
Participants, n=	41	105
Sufficient results	44 %	34 %

The low pass rate is probably due to the widely usage of presumably less robust Abs and the challenge for the laboratories to identify a reliable and consistent positive control with a low to moderate expression of CD99. Typically the laboratories, as recommended by many vendors, use a pPNET with a strong expression of CD99 as positive control. However, many normal cells, such as basal squamous epithelial cells, lymphocytes and endothelial cells, also express CD99. This may complicate the interpretation and cause the laboratories to calibrate their protocols in order to reduce or abolish this reaction and "improve the specificity". However, this may compromise the sensitivity, causing false negative reactions in tumours with a low CD99 antigen expression.

In run 12, NordiQC imprecisely stated that "no staining should be seen in the liver". While the liver cells are negative, it should be emphasized that the endothelial cells in the portal tracts and the sinusoids are positive.

Conclusion
The mAb clone 12E7 and the rmAb clone EPR3097Y are both recommendable Abs for CD99.
HIER in an alkaline buffer is mandatory to obtain an optimal result for both clones. Esophagus is recommended as positive control: The basal squamous epithelial cells must show an at least moderate distinct membranous staining while no cytoplasmic staining should be seen in the intermediate and superficial cells.

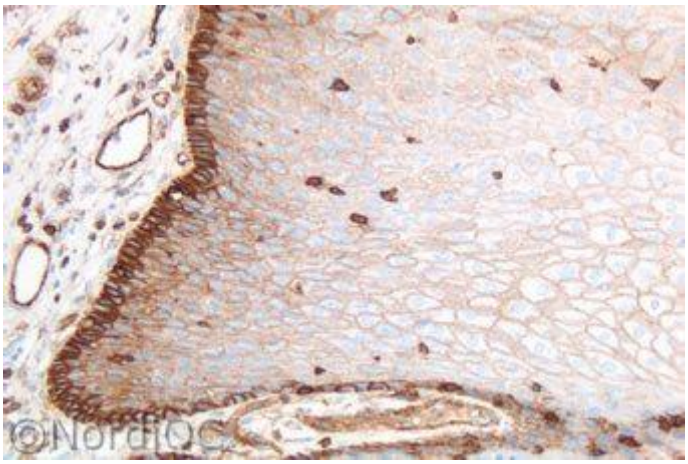


Fig. 1a
Optimal CD99 staining of the esophagus using the rmAb clone EPR3097Y optimally calibrated and with HIER in an alkaline buffer. The basal squamous epithelial cells show a moderate to strong, distinct membranous staining. Also lymphocytes and endothelial cells are strongly stained. Note the negative cytoplasmic reaction in the mature epithelial cells.

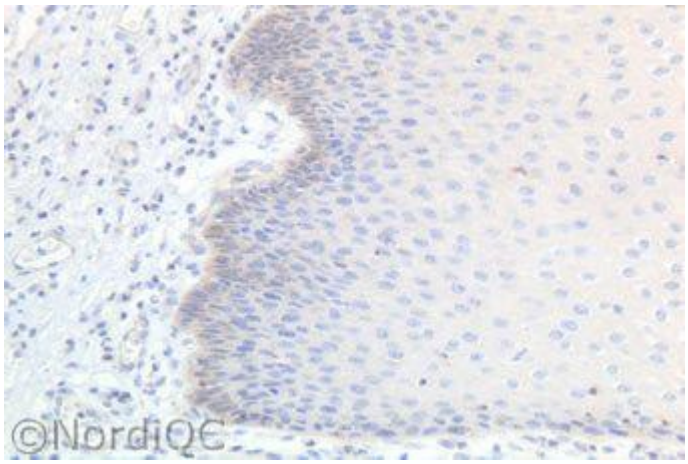


Fig. 1b
Insufficient CD99 staining of the esophagus using the mAb clone H036-1.1 - same field as in Fig. 1a. The basal squamous epithelial cells, lymphocytes and endothelial cells only show a weak and diffuse staining – also compare with Figs. 2b – 4b, same protocol.

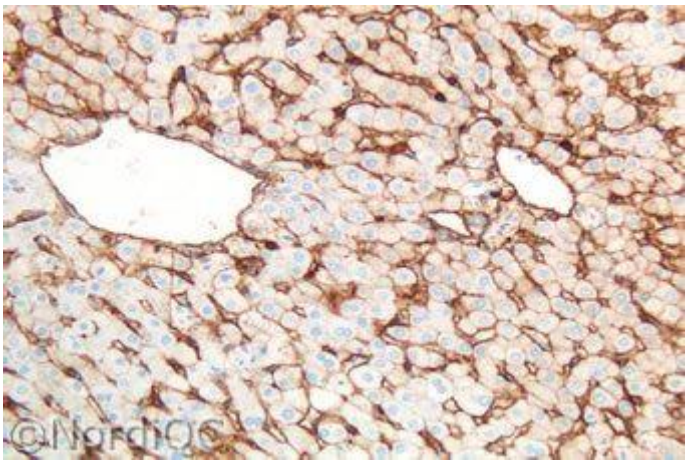


Fig. 2a
Optimal CD99 staining of the liver using same protocol as in Fig. 1a. Virtually all the endothelial cells in the central veins and in the sinusoids show a moderate-strong, distinct staining. No staining is seen in the liver cells.

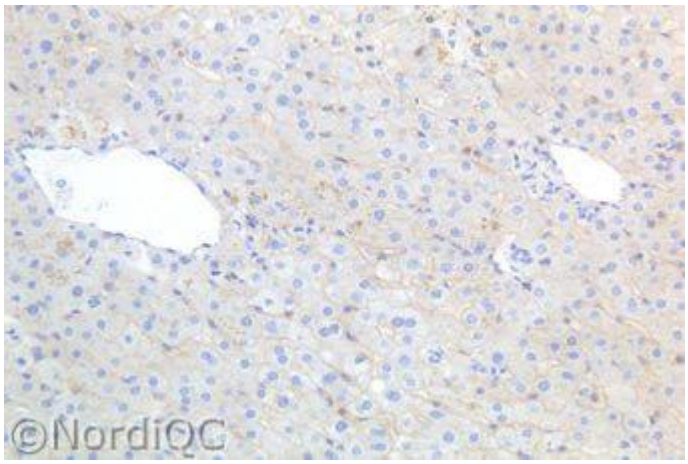


Fig. 2b
Insufficient CD99 staining of the liver using same protocol as in Fig. 1b - same field as in Fig 2a. The endothelial cells only show a weak or equivocal staining – also compare with Figs. 3b & 4b, same protocol.

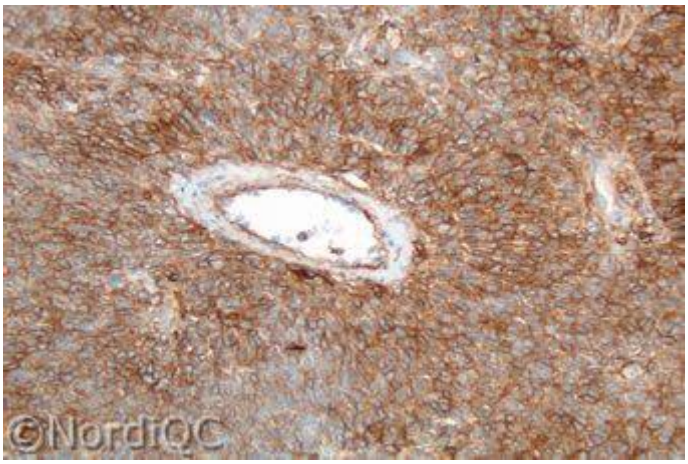


Fig. 3a
Optimal CD99 staining of the granulosa cell tumour using same protocol as in Figs. 1a & 2a. The majority of the neoplastic cells show a distinct membranous staining.

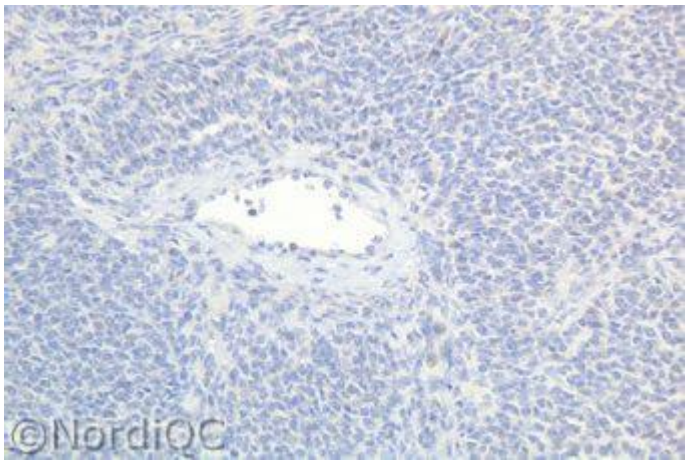


Fig. 3b
Insufficient CD99 staining of the granulosa cell tumour using same protocol as in Figs. 1b & 2b – same field as in Fig. 3a. Both the neoplastic and the endothelial cells are false negative.

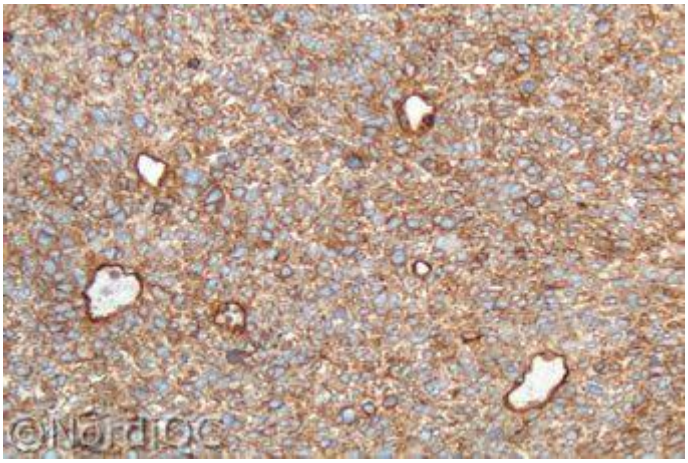


Fig. 4a
Optimal CD99 staining for CD99 of the pPNET (MB tissue no. 5) using same protocol as in Figs. 1a – 3a. Virtually all the neoplastic cells show a moderate, predominantly membranous staining. Scattered cells also show a cytoplasmic dot-like staining.

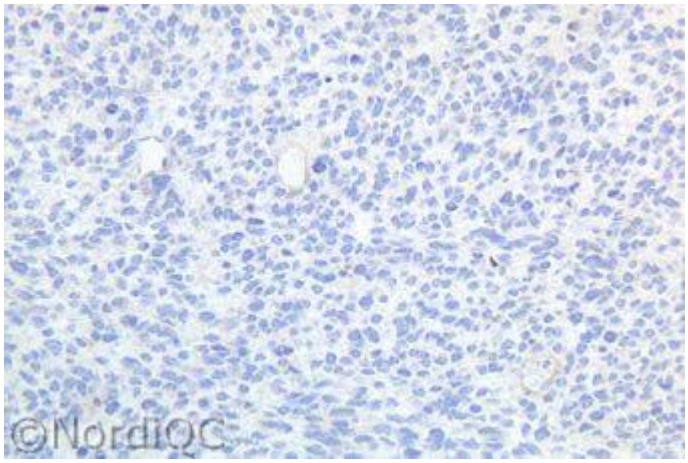


Fig. 4b
CD99 staining of the pPNET (MB tissue no. 5) using same insufficient protocol as in Figs. 1b – 3b. – same field as in Fig. 4a. Only the endothelial cells show a weak staining, while all the neoplastic cells are false negative.

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