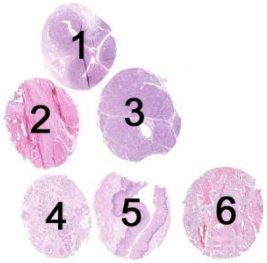
Assessment Run 29 2010
CK19

The slide to be stained for CK19 comprised:
1. Appendix, 2. Thyroid gland, 3. Pancreas, 4. Ductal breast carcinoma,
5. Esophagus, 6. Papillary thyroid carcinoma.
All tissues were fixed in 10% neutral buffered formalin for 24-48 h.



Criteria for assessing a CK19 staining as optimal included:

- A strong, distinct cytoplasmic reaction of virtually all the appendiceal surface epithelial cells, and at least a weak to moderate reaction of the epithelial cells in the basal part of the crypts.
- A strong, distinct cytoplasmic reaction of virtually all the epithelial cells of the large pancreatic ducts, while the epithelial cells of the intercalating ducts at least should show a weak to moderate cytoplasmic reaction.
- A strong cytoplasmic reaction of the majority of the basal squamous epithelial cells in the esophagus and a weak to moderate reaction of scattered intermediate epithelial cells.
- At maximum a weak to moderate reaction in scattered epithelial cells in the thyroid gland.
- A moderate to strong, distinct reaction of the majority of the neoplastic cells of the ductal breast carcinoma and the papillary thyroid carcinoma.

109 laboratories participated in this assessment. 69 % achieved a sufficient mark. In table 1 the antibodies (Abs) used and marks are summarized.

Table 1. **Abs and assessment marks for CK19, run 29**

Concentrated Abs	N	Vendor	Optimal	Good	Borderl.	Poor	Suff.[1]	Suff. OPS[2]
mAb[3)] clone **RCK108 56**	56 3 2 1 1	Dako Euro-Diagnostica BioGenex Abcam NeoMarkers	13	25	17	8	60%	72%
mAb clone **A53-B/A2.26**	4 1	NeoMarkers Cell Marque	3	2	0	0	100%	100%
mAb clone **b170**	7 1	Novocastra/Leica Vector	3	3	2	0	75%	100%
mAb clone **BA17**	2 1 1	NeoMarkers Dako Master Diagnostica	4	0	0	0	-	-
mAb clone **Ks19.1**	2	Biocare	2	0	0	0	-	-
mAb clone **K19.2**	2	NeoMarkers	0	1	1	0	-	-
pAb **RB-9021**	2	NeoMarkers	0	1	0	1	-	-
Ready-To-Use Abs								
mAb clone **A53-B/A2.26, 760-4281**	11	Ventana	8	2	0	1	91%	100%
mAb clone **A53-B/A2.26, 319M-17**	2	Cell Marque	2	0	0	0	-	-
mAb clone **RCK108, IR615**	9	Dako	0	5	4	0	56%	-
mAb clone **RCK108, RM-1902-R7**	1	NeoMarkers	0	1	0	0	-	-
mAb clone **Ks19.1, PM242**	1	Biocare	0	1	0	0	-	-

Total	109		34	41	24	10	-	-
Proportion			31%	38%	22%	9%	69%	-

1) Proportion of sufficient stains (optimal or good)
2) Proportion of sufficient stains with optimal protocol settings only, see below.
3) mAb: mouse monoclonal antibody, pAb: polyclonal antibody.

The following central protocol parameters were used to obtain an optimal staining:

Concentrated Abs
mAb clone **RCK108**: The protocols giving an optimal result were all based on HIER with either Tris-EDTA/EGTA pH 9 (5/17)*, Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako) (6/16), Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/1) or Citrate pH 6 (1/2) as the retrieval buffer. The mAb was typically diluted in the range of 1:20– 1:100 depending on the total sensitivity of the protocol employed. Using these protocol settings 26 out of 36 laboratories (72 %) produced a sufficient staining (optimal or good).
One lab used a combined epitope retrival by enzyme pre-treatment in Protease 3 (Ventana) and HIER in Cell Conditioning 1 (BenchMark, Ventana). The mAb dilution was 1:50.
* (number of optimal results/number of laboratories using this buffer)

mAb clone **A53-B/A2.26:** The protocols giving an optimal result were based on HIER with either Tris-EDTA/EGTA pH 9 (1/1), Bond Epitope Retrieval Solution 1 (Bond, Leica) (1/1) or Citrate pH 6 (1/3) as the retrieval buffer. The mAb was typically diluted in the range of 1:50– 1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings all of 3 laboratories produced a sufficient staining (all optimal).

mAb clone **b170:** The protocols giving an optimal result were based on HIER with Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/1), Cell Conditioning 1 (BenchMark, Ventana) (1/1) or Citrate pH 6 (1/1) as the retrieval buffer. The mAb was diluted in the range of 1:50– 1:300 depending on the total sensitivity of the protocol employed. Using these protocol settings all of 4 laboratories produced a sufficient staining.

mAb clone **BA17:** The protocols giving an optimal result were based on HIER with either Target Retrieval Solution pH 9 (EnVision FLEX TRS high pH, Dako)(1/1), Cell Conditioning 1 (BenchMark, Ventana) (1/1), EDTA/EGTA pH 8 (1/1) or Citrate pH 6 (1/1) 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 all of 4 laboratories produced a sufficient staining (all optimal).

mAb clone **Ks19.1:** The protocols giving an optimal result were based on HIER using Bond Epitope Retrieval Solution 2 (Bond, Leica) (1/1) as the retrieval buffer or enzymatic pre-treatment in Protease 1 (Ventana). 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 both of 2 laboratories produced an optimal staining.

Ready-To-Use Abs
mAb clone **A53-B/A2.26** (prod. no. 760-4281, Ventana): The protocols giving an optimal result were based on HIER using mild or standard Cell Conditioning 1, an incubation time of 16-48 min in the primary Ab and UltraView (760-500) or iView (760-091) as the detection system. Using these protocol settings all of 10 (100 %) laboratories produced a sufficient staining.

mAb clone **A53-B/A2.26** (prod. no. 319M-17, Cell Marque): The protocol giving an optimal result was based on HIER using mild Cell Conditioning 1, an incubation time of 16 min in the primary Ab and UltraView (760-500) as the detection system.

The most frequent causes of insufficient staining were:
- Too low concentration of the primary Ab
- Inappropriate epitope retrieval (e.g., all of 7 protocols based on enzymatic pre-treatment for the mAb clone RCK108 gave an insufficient result)
- Insufficient HIER – too short efficient heating time
- Omission of HIER.

In this assessment the prevalent feature of an insufficient staining was a too weak or completely false negative staining of structures expected to stain. Virtually all laboratories were able to demonstrate CK19 in high antigen expressing structures as the pancreatic ducts and the the ductal breast adenocarcinoma, whereas the basal and intermediate squamous epithelial cells of the esophagus and the papillary thyroid carcinoma expressing less CK19 was thus challenging and required an optimally calibrated protocol. The mAb clone RCK108 was the most commonly used Ab for CK19 and could only give a sufficient result if HIER was performed. All of 7 stains based

on enzymatic pre-treatment were insufficient (all other protocol settings were similar to protocols based on HIER giving sufficient results). The recommended protocol from Dako (the most used vendor) for the mAb clone RCK108 (as a concentrate) is based on proteolytic pre-treatment, whereas HIER is recommended when the clone is sold as a Ready-To-Use (RTU) format from same vendor!

The mAb clone A53-B/A2.26 appeared to be more robust than the mAb clone RCK108, as all of the laboratories produced a sufficient staining when the clone A53-B/A2.26 was applied with the optimal protocol settings for both the concentrated format and the RTU format (compared to 69 % for the mAb clone RCK108). The mAbs clone BA17 and Ks19.1 also appeared to be robust and superior to the mAb clone RCK108, even though the number of protocols was limited.

As control, esophagus displayed the most informative reaction pattern as a critical stain quality indicator for CK19. In the optimal protocols virtually all the basal squamous epithelial cells showed a moderate to strong distinct cytoplasmic staining reaction and - importantly - also scattered intermediate epithelial cells were demonstrated. In the insufficient stains deemed too weak the intermediate cells were negative and the basal cells only showed an equivocal or totally negative staining reaction.

Conclusion
The mAb clones RCK108, A53-B/A2.26, b170, BA17 and Ks19.1 are all recommendable Abs for CK19. Clone A53-B/A2.26 was in this assessment the most robust Ab. For all clones HIER should be used for an optimal performance. Esophagus is an appropriate control for CK19: Virtually all the basal squamous epithelial cells as well as scattered intermediate cells must show a distinct cytoplasmic staining.

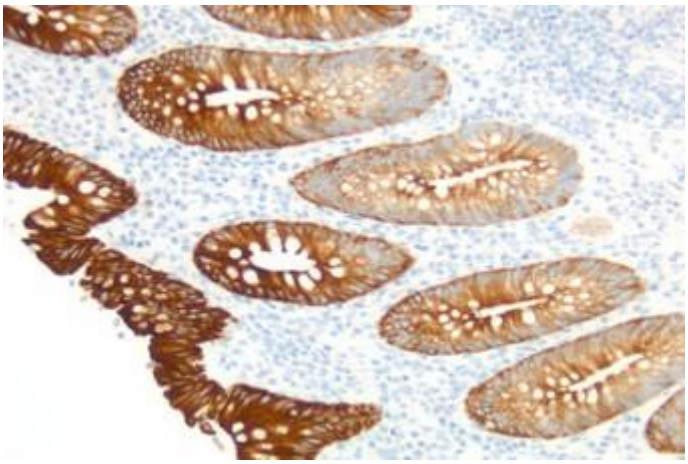


Fig. 1a
Optimal CK19 staining of the appendix using the mAb clone A53-B/A2.26. The surface columnar epithelial cells show a strong cytoplasmic staining, while the columnar epithelial cells in the basal parts of the crypts show a weak to moderate staining. No background staining is seen.

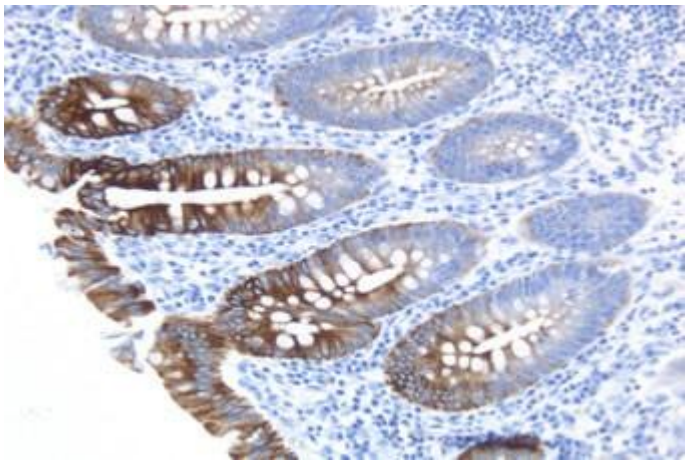


Fig. 1b
Insufficient CK19 staining of the appendix using the mAb clone RCK108 with proteolytic pre-treatment. Only the luminal columnar epithelial cells show a moderate cytoplasmic staining, while virtually no staining is seen in the basal part of the crypts – also compare with Figs. 2b - 4b same protocol.

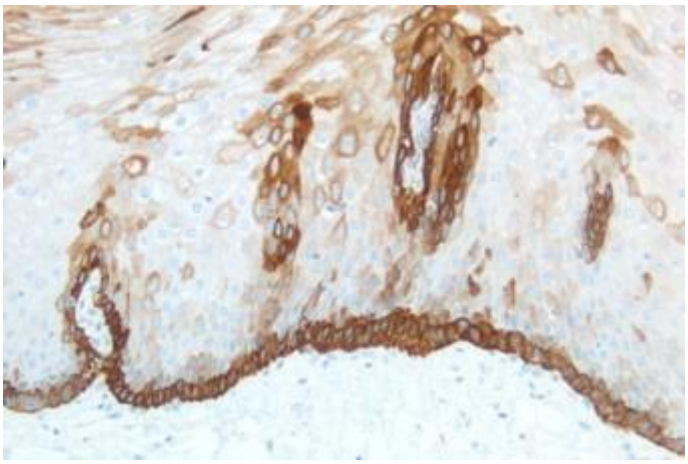


Fig. 2a
Optimal CK19 staining of the esophagus using same protocol as in Fig. 1a. The majority of the basal squamous epithelial cells show a strong cytoplasmic reaction. A weak to moderate reaction is seen in scattered suprabasal epithelial cells.

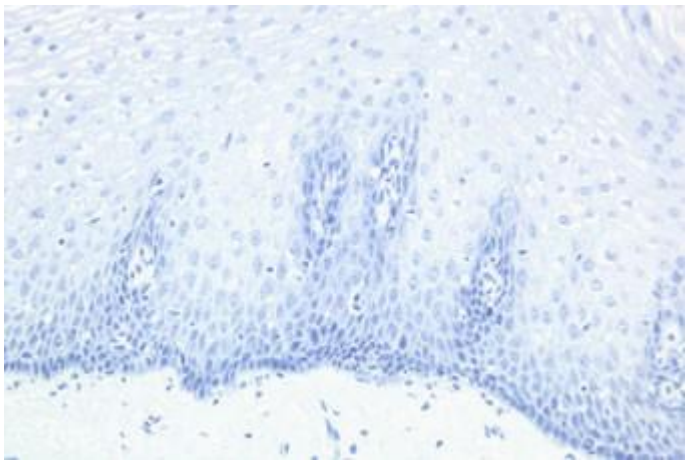


Fig. 2b
Insufficient CK19 staining of the esophagus using same protocol as in Fig. 1b. No staining in the squamous epithelial cells is seen. This staining pattern typically appeared, when proteolytic pre-treatment was applied for the mAb clone RCK108.

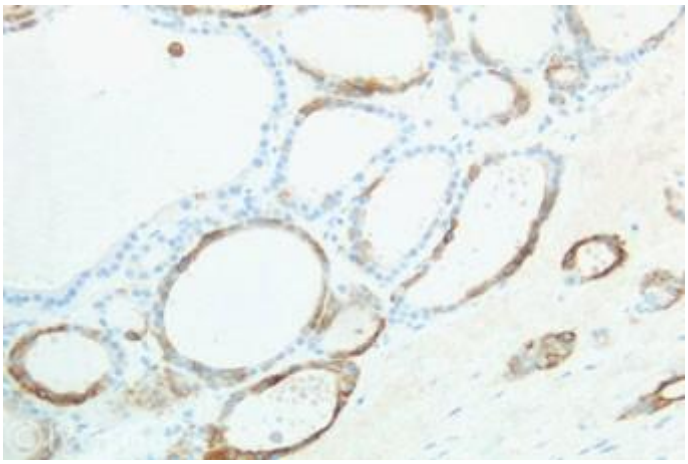


Fig. 3a
Optimal CK19 staining of the thyroid gland using same protocol as in Figs. 1a & 2a. Scattered epithelial cells show a weak to moderate cytoplasmic staining. Compare with the staining reaction in the papillary thyroid carcinoma in Fig. 4a.

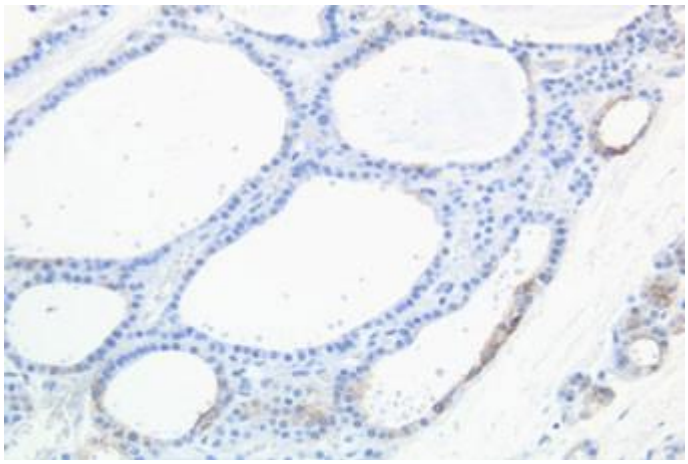


Fig. 3b
CK19 staining of the thyroid gland using the same insufficient protocol as in Figs. 1b, 2b & 4b. Scattered epithelial cells show a weak to moderate cytoplasmic staining. Also compare with the staining reaction in the papillary thyroid carcinoma in Fig. 4b.

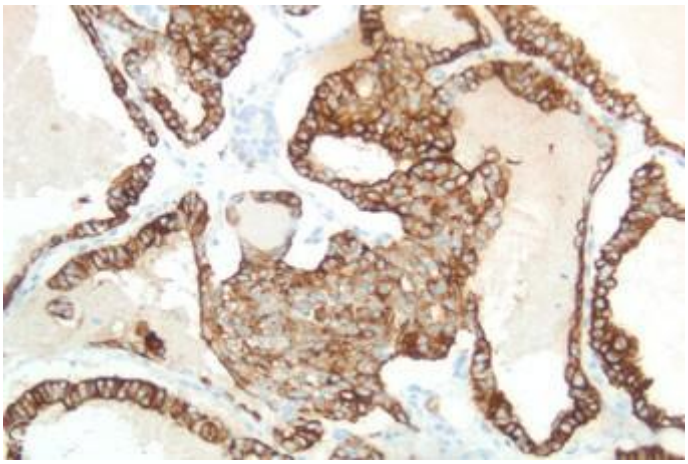


Fig. 4a
Optimal CK19 staining of the papillary thyroid carcinoma using same protocol as in Figs. 1a - 3a. Virtually all the neoplastic cells show a moderate to strong cytoplasmic staining. No background reaction is seen.

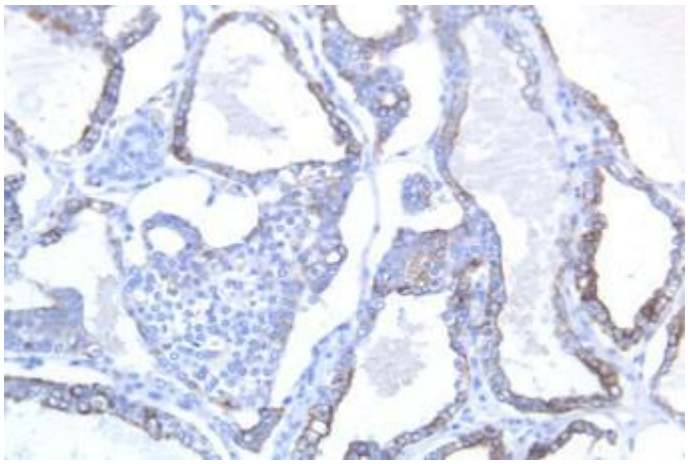


Fig. 4b
Insufficient CK19 staining of the papillary thyroid carcinoma using same protocol as in Figs. 1b – 3b. Only scattered neoplastic cells show a weak staining.

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