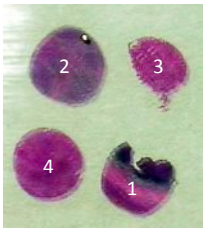
The slides to be stained for CK(-pan) comprised :



1. Appendix, 2. Infiltrating duct carcinoma, 3. Kidney and 4. Liver.

All tissues sent were fixed in 10% neutral buffered formalin.

Criteria for assessing staining as optimal were:

- 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 moderate to strong, distinct reaction of the majority of the neoplastic cells of the ductal breast carcinoma
- Moderate to strong cytoplasmic staining of distal tubular epithelial cells. Stronger cytoplasmic staining with membrane accentuation of proximal tubular epithelial cells. Strong staining of cells lining Bowmann capsule.
- A strong distinct cytoplasmic reaction of virtually all bile duct epithelial cells and at least moderate cytoplasmic staining with membrane accentuation of hepatocytes.

Participants' overall performance was assessed based on following criteria:

Selection of proper antibody, label, integrity of sections, artefacts affecting overall staining in all tissues, intensity of staining reaction in each tissue in the set, proportion of staining reaction in each tissue in the set, non-specific staining reaction in and background staining.

Participants' performance specific for the antibody related parameters was assessed separately based on following criteria:

intensity of staining reaction in each tissue in the set and proportion of staining reaction in each tissue in the set.

Participation:

8 laboratories participated

Results:

The results were assessed based on following cut offs:

	Cut off	No. of labs (total 8)
Optimal	>90%	3
Good	>80%	3
Borderline	>70%	0
Poor	</=70%	2

Antibody details and performance analysis:

Clone	RTU	Conc	Vendor	N	Optimal	Good	Borderline	Poor
mAb clone cocktail AE1/AE3	5		Dako	3	1	2		
			Ventana	1				1
			Cellmarque	1	1			
		3	Dako	2	1	1		
			Biocare	1				1

The causes of insufficient staining were:

- Inappropriate epitope retrieval (seen as weak staining across all sections)
    - One of the labs used high temperature for longer time
    - One of the labs used enzyme as retrieval method
    - Dewaxing temperatures were faulty in case of 2 laboratories (more than 75 degree)

Conclusions:

- Clone AE1/AE3 cocktail is a good clone
- RTU or concentrated antibody does not change performance
- Appropriate epitope retrieval is the most important step. Both the poor outcomes were seen with that.
- Enzyme is not a proper method for epitope retrieval as there was poor outcome in the run. The one using enzyme as retrieval method used dilution of 1:50 while the ones using HIER used dilutions of 1:250 and 1:300.
- Liver and appendix are appropriate control tissues. Vast majority of hepatocytes should show cytoplasmic staining with membrane accentuation. The bile ducts should show more intense staining. The appendicular crypts should stain intensely while the lymphoid and muscle tissue should serve as good negative control.

Critical parameter analysis:

Critical parameters were assessed based purely on the antibody performance and protocol setting (i.e staining intensity and proportion only while excluding integrity of sections, artefacts, background etc.)

Total 5 laboratories had optimal staining performance in critical parameter analysis. Following parameters were central to optimal staining performance:

RTU antibodies (4/5):

3 used manual method while 1 used automation.

Optimal staining results obtained used HIER as retrieval method (4/4) in Tris-buffered saline at pH 9.0 (3/4) or low pH (1/4).

The efficient heating time has been 90 to 95 degrees C for 20-25 minutes. Microwave and pressure cooker were used by 1 each. 1 used Dako Decloaking chamber while the automated one used Leica Bond system.

Primary antibody incubation time was 60 min in 3 while 30 minutes in 1 out of 4.

Secondary were all polymer based with incubation time of 30 minutes or 60 minutes (Dako, Cell marquee, Biocare and Bond).

One lab used primary antibody 'expired' in year 2011 and was the best performer out of the RTU group!

Concentrated antibodies (1/5):

It used manual method.

HIER as retrieval method in Tris-buffered saline at pH 9.0

The efficient heating time has been 100 degrees C for 20 minutes. Microwave was used by 1 each. 1 used.

Primary antibody incubation time was 30 min.

Secondary was Dako Envision, polymer based with incubation time of 30 minutes.

It used primary antibody 'expired' in year 2010!

Recommended protocol for CK-PAN (AE1/AE3) for RTU

Obtained in General Module, run 1

Primary antibody

Clone	AE1/AE3
Producer	Cell Marque, Cat No. 313M-17
Product no. (Lot no.)	1205907D
Dilution	RTU
Diluent buffer and additive(s)	NA
Incubation time / temperature	60 min./RT

Epitope retrieval, HIER

Device	Microwave oven
Buffer, pH	Tris-EDTA buffer, pH 9
Warm-up / heating max / resting time	AT 640 WATT 5 MIN X 2 CYCLE AND 800 WATT 3 MIN X 1 CYCLE

Visualization system

Method	2-step polymer conjugate
Producer, product no.	HIGH DEFINITION DETECTION POLYMER SYSTEM, CELL MARQUE, CAT NO 954D-50, BATCH NO 1330406
Incubation time / temperature	60 min./RT

Chromogen

Type	DAB
Enhancement, type	Nil

Immunostainer

Type	Manual

Abbreviations HIER: Heat induced epitope retrieval, RT: Room temperature

Steps in brief:

DEWAXING- REHYDRATION- ANTIGEN RETRIEVAL- WASH (BUFFER)- BLOCKING- PRIMARY ANTIBODY INCUBATION- WASH (BUFFER)- AMPLIFIER INCUBATION- WASH (BUFFER)- SECONDARY ANTIBODY INCUBATION- WASH (BUFFER)- DAB CHROMOGEN- DW WASH- COUNTER STAIN- WASH TAP (WATER)- DEHYDRATION- MOUNT

Recommended protocol for CK-PAN (AE1/AE3) for concentrated antibody

Obtained in General Module, run 1

Primary antibody

Clone	AE1/AE3
Producer	Dako
Product no. (Lot no.)	00007993
Dilution	1:250
Diluent buffer and additive(s)	Dako antibody diluents with twin20
Incubation time / temperature	30 min./RT

Epitope retrieval, HIER

Device	Microwave oven
Buffer, pH	Tris-EDTA buffer, pH 9
Warm-up / heating max / resting time	10 min. warm up then 20 min 95 to 100 degree C

Visualization system

Method	1-step polymer conjugate
Producer, product no.	Dako Envision plus
Incubation time / temperature	60 min./RT

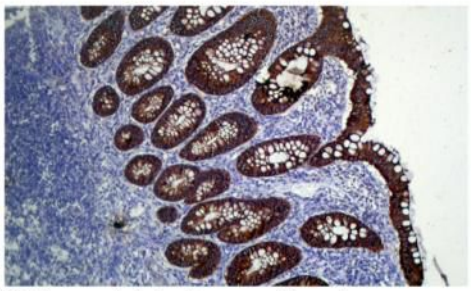
Chromogen

Type	DAB
Enhancement, type	Copper sulphate

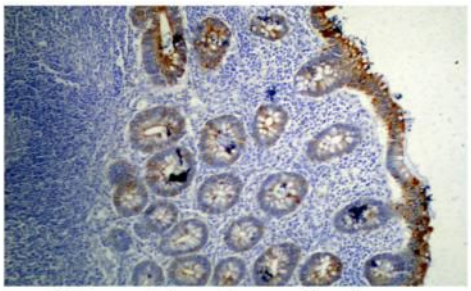
Immunostainer

Type	Manual

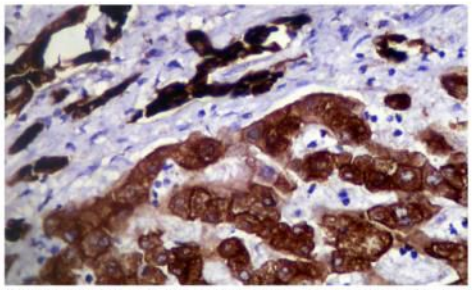
Abbreviations HIER: Heat induced epitope retrieval, RT: Room temperature



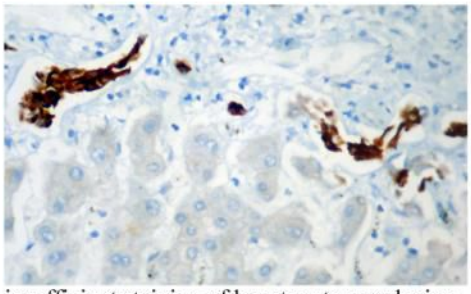
Optimum staining in appendix, using clone AE1/AE3, HIER at pH 9.0 in Tris-EDTA buffer



Insufficient staining of appendix using enzyme for pre-treatment.

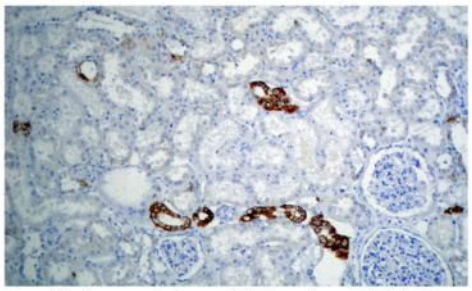


Optimum stainining of hepatocytes and bile ducts using concentrated antibody AE1/AE3. Note the cytoplasmic staining of hepatocytes, less intense than the bile ducts

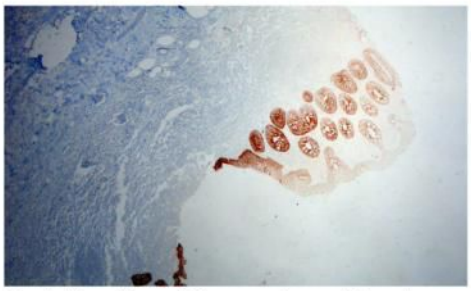


insufficient staining of hepatocytes producing no staining at all while the bile ducts are stained

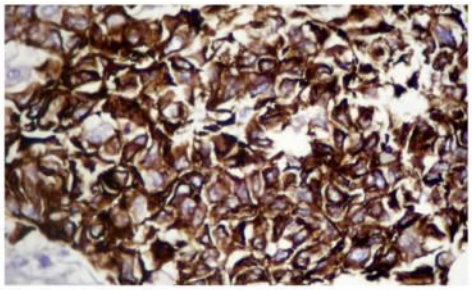
P.T.O.



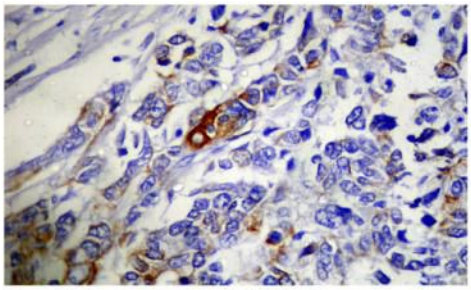
Insufficient staining of kidney by AE1/AE3 using HIER. The temperature has been sub-optimal for effective HIER.



Probable effect of slant on the staining that can be seen as relatively unstained area in this suboptimally stained section of appendix



Optimal staining of IDC using concentrated antibody AE1/AE3, HIER at 95 C for 20 min



Insufficient staining of IDC using enzymatic pre-treatment. The clone used is AE1/AE3.