General module. Cycle 1, Run 2

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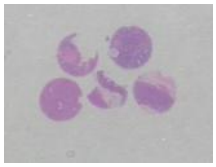
May 2015



The slides to be stained for CK7 comprised :

1. ~~Liver~~
2. Colonic adenocarcinoma
3. Infiltrating duct carcinoma of breast
4. Gastric mucosa
5. Kidney tissue
6. Colonic mucosa

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

Criteria for assessing staining as optimal were:

Tissue	Reaction pattern
Liver	Liver tissue was exhausted in some of the sections and hence **NOT CONSIDERED PART OF ASSESSMENT**. ~~**A strong and distinct cytoplasmic staining of bile duct epithelial cells. The hepatocytes should be negative, except for few scattered ones around portal areas.**~~
Colonic adenocarcinoma	**No** staining of the tumor cells or any cells in the sections.
Breast-IDC	A **strong** and distinct cytoplasmic staining of tumor cells. **Majority of the tumor cells should be stained.**
Gastric mucosa	An at least **weak to moderate predominantly cytoplasmic staining** reaction of a distinct subset of luminal foveolar epithelial cells of the gastric corpus mucosa. There should not be any staining of the chief cells.
Kidney tissue	A **moderate to strong, distinct cytoplasmic staining reaction of virtually all epithelial cells of the renal collecting ducts** and the scattered epithelial cells in the Bowman capsule.
Colonic mucosa	**No staining** of any cell in colonic mucosal piece

Participants' overall performance was assessed based on following criteria:

1. Selection of proper antibody
2. Label
3. Integrity of sections
4. Artifacts affecting overall staining in all tissues
5. Intensity of staining reaction in each tissue in the set.
6. Proportion of staining reaction in each tissue in the set
7. Non-specific staining reaction and background staining.

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

1. **Intensity** of staining reaction in each tissue in the set and
2. **proportion** of staining reaction in each tissue in the set.

The background and non-specific staining is usually the attribute of labeling steps/dilution of antibody/blocking steps etc. Therefore, we have restricted the performance character of the antibody to be judged by intensity and proportion of staining reaction. This may help one choose proper antibody clone/vendor.
However the performance of the laboratory as far as technique of IHC is concerned, should be judged by overall score.

Participation:

Fourteen, 14 laboratories participated

Results:

The results were assessed based on following cut offs for the overall score:

	Cut off	No. of labs (total)
Optimal	>90%	9
Good	>80%	0
Borderline	>70%	2
Poor	</=70%	3

Antibody details and performance analysis based on critical parameter score:

RTU	Conc	Vendor	N	Clone	Optimal	Good	Borderline	Poor
12		Dako, IS619	6	OV-TL 12/30	6 (100%)			
		Biogenex - AM255-5M	3	OV-TL 12/30		1 (33%)		2 (66%)
		Cell Marque 307M-97	1	OV-TL 12/30				1 (100%)
		Leica Bond Max	1	OV-TL 12/30				1 (100%)
		Thermoscientific, MS-1352-R7	1	OV-TL 12/30				1 (100%)
	2	Dako, M7018	1	OV-TL 12/30	1 (100%)			
		Biocare, CRM339A	1	BC1, rabbit				1 (100%)

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Comments:

1. **Which clone has the best outcome?**

   Out of 14 participants 13 used clone **OVTL 12/30**. It is the most common clone and hence best outcome are observed with that clone. The only lab that used clone BC1 from Biocare had poor overall and antibody specific performance.

2. **Which vendor has the best performance?**

   Within the group of 13 labs that used clone OVTL 12/30, the results were much superior with **Dako** antibody, IS619 RTU (6 labs). Equally good performance was achieved with Dako concentrated antibody Dako M7018 (1 lab).

3. **What was better, RTU or Concentrated?**

   Only 2 laboratories used concentrated antibody. One of them used Dako antibody and had one of the best performances. The other laboratory used clone BC1 and fared poorly in this assessment.

   The RTU group has clear bias towards the antibody supplier. Six laboratories used Dako antibody and all had optimum performance. The other six used same clone from different suppliers. None of them had optimum performance. Only one lab had good performance while the rest 5 were poor. Even the one with good performance had 33% pass rate for that vendor. (Rever the table above)

   The typical protocol employed, irrespective of RTU or concentrated, had HIER with MWO in buffer at alkaline pH of 9.0 (only one lab had pH of 8.4).

4. **What is the best dilution ratio of the primary antibody?**

   The one that used Dako antibody had best performance and used dilution of **1:300** in Dako antibody diluent. ***They used expired antibody. The 'expiry date' was August-2010!***

5. **Has dewaxing temperature to do something with staining reaction?**

   I could see range of dewaxing temperature in this run. It was 60 degree to 76 degree C. Total 9 labs had dewaxing temperature of equal or less than 70 C. 8 out of 9 passed it with optimum staining. Five laboratories used temperature above 70 C. Only one laboratory had optimum staining (in automated setting). This clearly indicates that **dewaxing temperature above 70 C is detrimental to overall staining reaction**. In this run melting point of the wax used was 58 to 60 C.

6. **Is the pH of retrieval buffer important?**

   Yes. Only two laboratories had acidic pH of the retrieval buffer. Both had poor antibody specific performance. All 9 laboratories with optimum performance used buffer at alkaline pH. 3 laboratories with suboptimal performance did use alkaline pH, however they had other factors also that were responsible for poor outcome.

   Alkaline pH is the most suitable for this marker.

7. **What is the best epitope retrieval method?**

   HIER was used by all laboratories.

8. **Which technique was better, manual or automated?**

   Manual technique was used by 10 labs and pass rate was 70% (7 out of 10 labs had overall and antibody specific score either optimum or good). The best scores were all achieved with manual technique.

   The automated technique was used by 4 labs and had pass rate of 50%. 3 out 4 labs used Ventana Benchmark (XT,XT and GX) platform and 1 used Leica platform. Both the labs using XT had optimum performance. The one using GX and the other one using Leica platform had poor performance.

9. **In the poor performance cases, what is more responsible, antibody clone or other factors?**

   All except one lab used clone OVTL 12/30 in this run. Rather than the clone, ***vendor statistics*** were noteworthy in this run. All those with borderline or poor performance had vendor other than Dako. It occurred with both RTU and Concentrated, automated and manual, and at pH range of 6.0 to 9.1. This may indicate that ***vendor selection is an independent parameter affecting overall staining***.

   The other important factor was ***dewaxing temperature and pH of retrieval buffer*** as elucidated above.

10. **Which antibody clones had poor performance?**

    Only 1 out of 14 labs used clone BC1. No conclusion could be derived on this.

11. **What are the most common causes of insufficient staining in the present run?**

a. Poor vendor selection
b. Higher dewaxing temperature above 70 C
c. HIER buffer at acidic pH

12. What is the best control material for this marker?

Recommended protocol for CK7 for RTU

Obtained in General Module, Run 2

Primary antibody

Clone	OVTL 12/30
Producer	Dako IS619
Product no. (Lot no.)	20010274
Dilution	RTU
Diluent buffer and additive(s)	NA
Incubation time / temperature	30 min./RT

Epitope retrieval, HIER

Device	Manual
Buffer, pH	Tris-buffered saline, pH 9.2
Peak temperature and time	96 C for 20 min

Visualization system

Method	Polymer conjugate
Producer, product no.	DAKO - REAL - EnVision system - DAKO - K5007- Lot. No. 20015510
Incubation time / temperature	30 min./RT

Chromogen

Type	DAB
Enhancement, type	Nil

Immunostainer

Type	Manual

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

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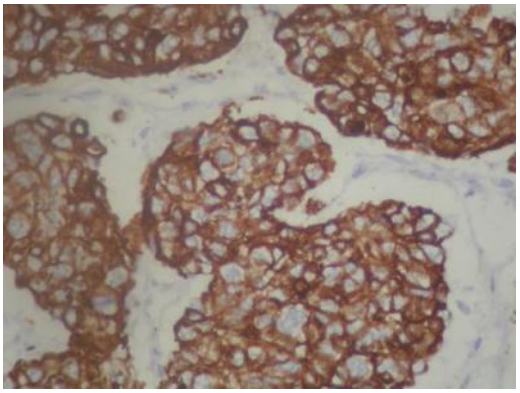

Fig.1: Optimum staining of IDC with Dako clone OVTL12/30, RTU, HIER at alkaline pH

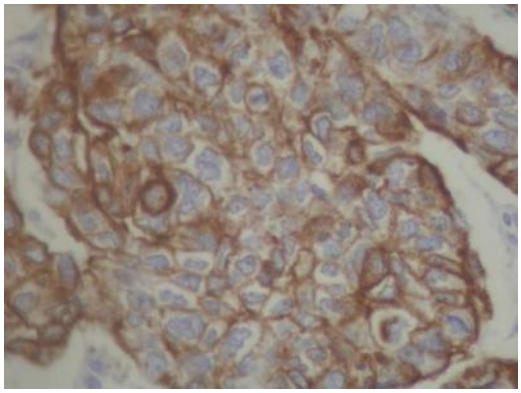

Fig.2: IDC, suboptimal staining reaction with Biogenex clone OVTL12/30,RTU, HIER at alkaline pH

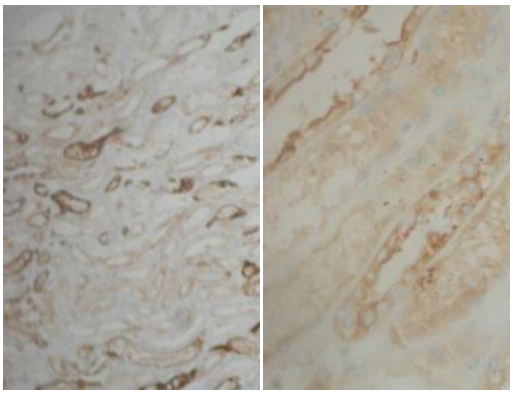

Fig.3: Suboptimal staining reaction using Dako IS619 RTU in automated Benchmark Ventana platform by two laboratories. Tubules are stained, however there is background staining.

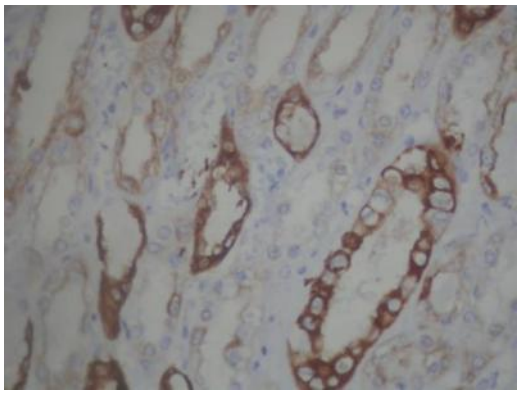

Fig.4: Optimum staining of renal tubular epithelial cells using Dako M0718, concentrated antibody in 1:300 dilution with HIER at pH 9.0 in MWO.

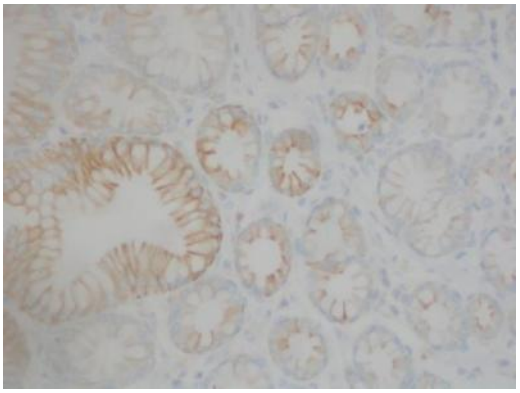

Fig.5: Optimum staining of gastric foveolar cells. There is weak staining of foveolar epithelial cells without staining of the chief cells, in a subset of cells only.

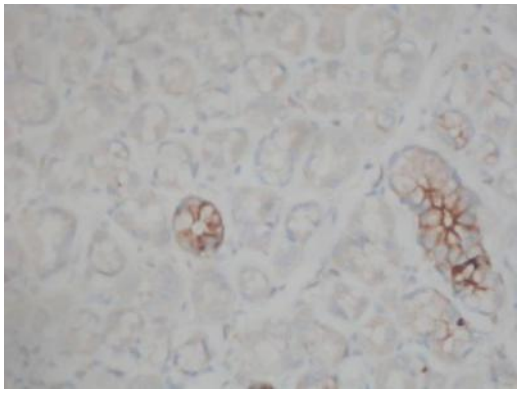

Fig.6: Suboptimal staining of gastric tissue. Note the less number of cells getting stained.