General module. Cycle 1, Run 2

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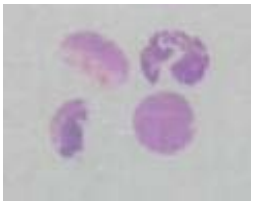
May 2015



The slides to be stained for CK20 comprised :

1. Colonic adenocarcinoma
2. Gastric mucosa
3. Appendix
4. Urothelial carcinoma

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

Criteria for assessing staining as optimal were:

Tissue	Reaction pattern
Colonic adenocarcinoma	A moderate to strong, distinct cytoplasmic staining reaction in virtually all the neoplastic cells of the colon adenocarcinoma
Gastric mucosa	An at least moderate, distinct cytoplasmic staining reaction of the majority of the foveolar epithelial cells of the stomach
Appendix	A strong, distinct cytoplasmic staining reaction of all the surface epithelial cells of the appendix and at least a weak to moderate staining reaction in most crypt cells
Urothelial carcinoma	An at least weak to moderate, distinct cytoplasmic staining reaction in the majority of the neoplastic cells of the urothelial carcinoma

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.

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.

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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%	2
Good	>80%	0
Borderline	>70%	5
Poor	</=70%	7

Antibody details and performance analysis based on critical parameter score:

RTU	Conc	Vendor	N	Clone	Optimal	Good	Borderline	Poor
11		Dako, IS777	5	Ks20.8			2 (40%)	3 (60%)
		Biogenex	1	Ks20.8				1 (100%)
		Biogenex	2	EPR1622Y	1 (50%)			1 (50%)
		Cell Marque	1	Ks20.8			1 (100%)	
		Leica Bond	1	Ks20.8				1 (100%)
		Thermoscientific	1	Ks20.8				1 (100%)
	3	Dako, M7019	1	Ks20.8			1 (100%)	
		Biocare, CM062A	2	Ks20.8	1 (50%)		1 (50%)	

Comments:

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

   This assessment saw overall poor performance. Only 2 laboratories had optimum outcome. One of them used Biogenex clone EPR1622Y, cat no. AM2550215, RTU. The other laboratory used concentrated clone Ks20.8 from Biocare CM062A.

   Clone EPR1622Y appears to be newer clone. The last NordiQc run of CK20 had 196 participants where this clone had not appeared. Though only one laboratory has used it, this clone appear to be the best in current run.

   Practically all the laboratories were able to demonstrate colonic adenocarcinoma and appendicular staining reactions. However, the staining of Urothelial carcinoma was the most challenging. Only two laboratories could achieve that.

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

   As majority of the laboratories had poor or borderline performance, no conclusions could be derived on this. Though Dako (Clone Ks20.8) was the most prevalent vendor, best results were obtained with Biocare clone Ks20.8 and Biogenex clone EPR1622Y.

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

   Of the two laboratories having optimum staining, one used RTU and the other used concentrated antibody.

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

   The one with optimum performance using concentrated antibody had dilution of 1:100.

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

   Both the laboratories with optimum performance had dewaxing temperature between 60 and 70 C. This is compatible with the observation (in CK7 overall analysis of this run) that dewaxing temperature between 60 C and 70 C is optimum.

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

   Yes.

   Both the laboratories with optimum performance had alkaline pH. Importantly, clone EPR1622Y (supposed to be the best) was used by two laboratories. The one that used pH of 6.0 for antigen retrieval had poor performance while the one using alkaline pH had optimum performance.

   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?**

   Automated and manual techniques were used by each of the two laboratories having optimum performance analysis.

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

   Poor antibody selection was the most prevalent feature of suboptimum performance. The most prevalent clone in this run was Ks20.8. However, majority of them failed. The one having optimum performance using this clone had vendor other than Dako (Biocare). This means that vendor selection is equally important as the clone.

Most laboratories failed staining the Urothelial carcinoma. One may consider using that as 'critical' control in their in-house quality control.

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

    Ks20.8 had poor performance in this run. This was recommended clone in NordiQc run of 2012. It is most widely used clone as well. However, this run shows different statistics.

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

    a. Poor clone and 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?**

    Colonic adenocarcinoma is the most suitable control. One may incorporate Urothelial carcinoma as critical tissue because staining Urothelial carcinoma is challenging for this marker.

Recommended protocol for CK7 for RTU

Obtained in General Module, Run 2

Primary antibody

Clone	EPR1622Y
Producer	Biogenex
Product no. (Lot no.)	AM2550215
Dilution	RTU
Diluent buffer and additive(s)	NA
Incubation time / temperature	60 min./RT

Epitope retrieval, HIER

Device	Manual
Buffer, pH	Tris-buffered saline, pH 9.0
Peak temperature and time	NA (using pressure cooker)

Visualization system

Method	Polymer conjugate
Producer, product no.	Labvision
Incubation time / temperature	15 min./RT

Chromogen

Type	DAB
Enhancement, type	Nil

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Immunostainer

Type Manual

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

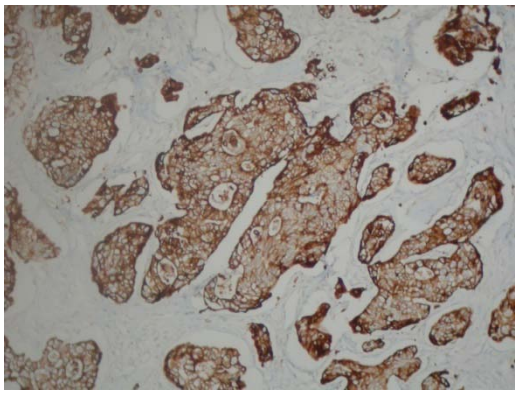

Fig.1: Optimum staining reaction of colonic adenocarcinoma case using Biocare clone Ks20.8 in Automated Ventana system at alkaline pH

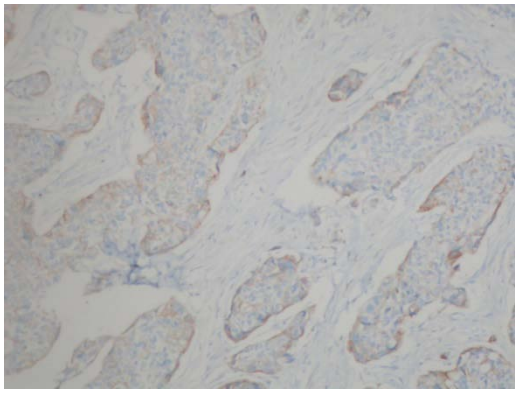

Fig.2: Poor staining of the same case using Thermo antibody clone Ks20.8 in Ventana system at alkaline pH.

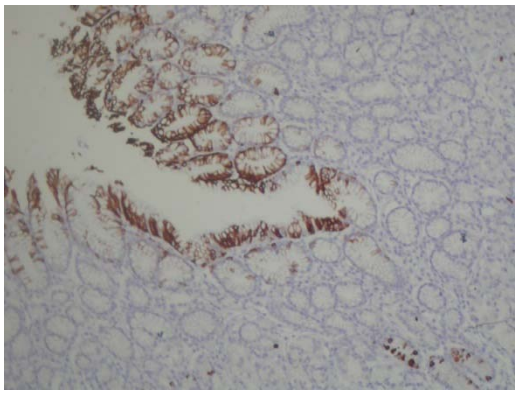

Fig.3: Optimum staining of gastric mucosa using Dako clone Ks20.8 at alkaline pH. The same lab failed to stain urothelial carcinoma case.

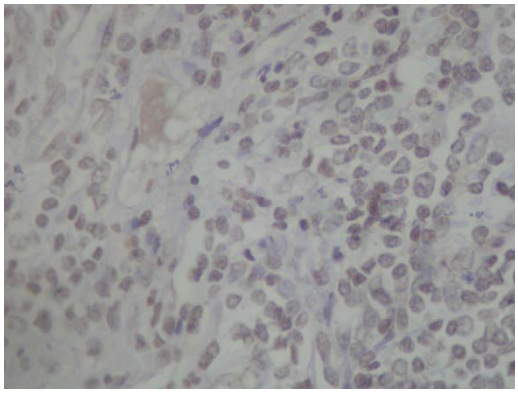

Fig.4: Non-specific staining of leukocytes using Biocare clone Ks20.8 at pH 6.2

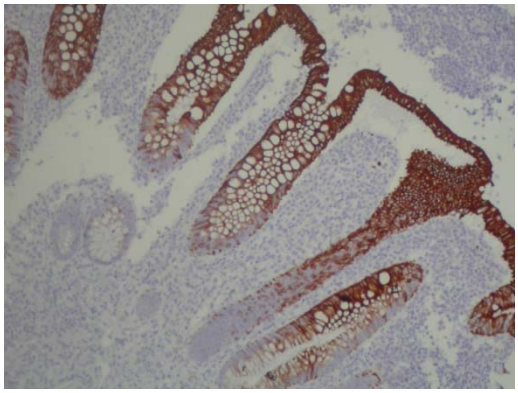

Fig.5: Optimum staining of appendicular crypts. *

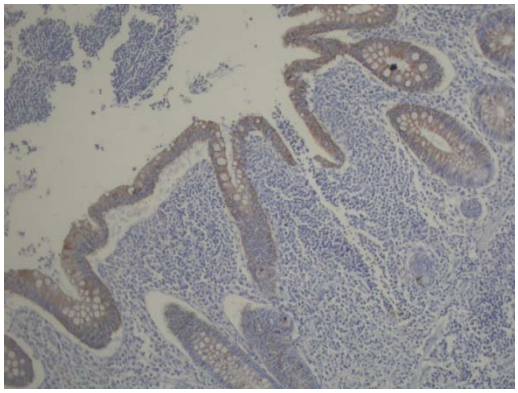

Fig.6: Poor staining of the crypts using biogenex clone EPR1622Y at pH 9.0

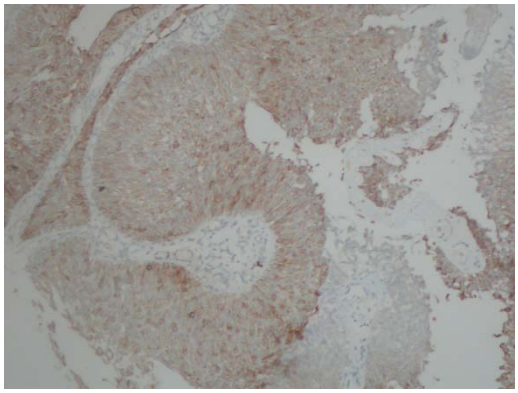

Fig.6: Optimum staining of urothelial carcinoma using Biocare clone Ks20.8 in automated Ventana Benchmark system using CC1 retrieval buffer pH 8.4

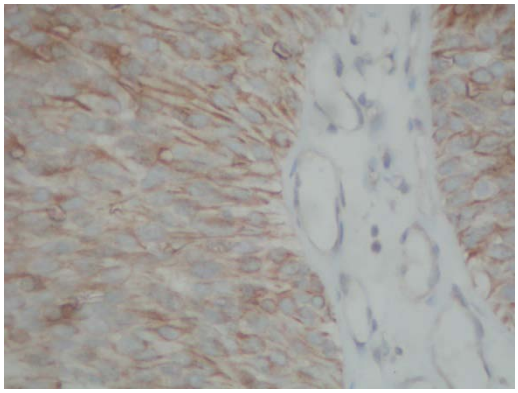

Fig.7: Same case as Fig.6

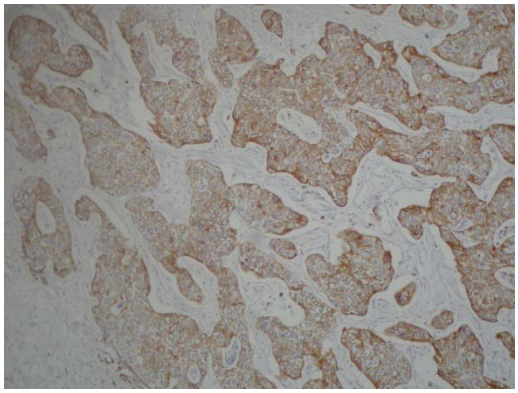

Fig.8: The only other laboratory that could stain urothelial carcinoma used Biogenex clone EPR1622Y

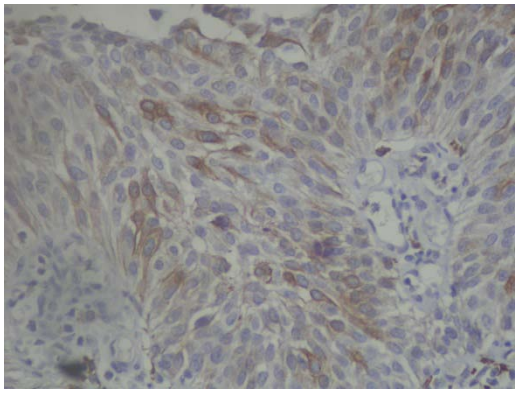

Fig.9: Same case as Fig.8. Note the weaker staining compared to the case in Fig.6,7