General module. Cycle 1, Run 2

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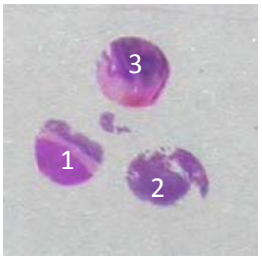
May 2015



The slides to be stained for CD3 comprised :

1. Colonic mucosa,
2. Tonsil, and
3. PTCL

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

Criteria for assessing staining as optimal were:

Tissue	Reaction pattern
Colonic mucosa	A moderate to strong, distinct, predominantly membranous staining reaction of the intra-epithelial T-cells in the colon mucosa.
Tonsil	A moderate to strong, distinct, predominantly membranous staining reaction of all T-cells both in the interfollicular T-zones and in the germinal centres of the tonsil.
PTCL	An at least weak to moderate, distinct, predominantly membranous staining reaction of the majority of the neoplastic T-cells in the two T-cell lymphomas.
No staining of other cells. Especially the B-cells in the tonsil should be negative.	

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 (**critical score**). 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

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Results:

The performance of the group was averaging 76% which should be considered suboptimum. The results were assessed based on following cut offs for the overall score:

	Cut off	No. of labs (total)
Optimal	>90%	5
Good	>80%	3
Borderline	>70%	2
Poor	</=70%	4

Antibody details and performance analysis based on critical parameter score:

RTU	Conc	Vendor	N	Clone	Optimum	Good	Borderline	Poor
11		Dako, A0452/IS503	6	Rabbit polyclonal	6 (100%)			
		Biogenex, AM258-5M	1	UCHT1	X	X	X	X
		Biogenex, AM322-5M	1	PS1	1 (100%)			
		Cell Marque	1	Rabbit polyclonal				1 (100%)
		Leica Bond	1	PS1				1 (100%)
		Path n situ	1	EP41	1 (100%)			
	3	Dako, A0452	1	Rabbit polyclonal	1 (100%)			
		Biocare, CM062A	2	EP41		1 (50%)		1 (50%)

** Clone UCHT1 is intended for use before paraffin embedding, most ideally in setting of frozen section.*

Comments:

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

   Rabbit polyclonal antibody from Dako (A0452) had the best performance, in both RTU and concentrated formats. Rabbit polyclonal antibody from Cell Marque has poor performance. However, it is due to heavy background staining in case of the sole laboratory using this. Dilution of RTUs is not recommended. However, at times sensitive protocol employed by laboratories necessitates dilution of even RTUs also.

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

   -

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

   Both had equal proportion of result categories, though there were more users of RTUs (11) than Conc. (3).

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

   The best performing Dako rabbit polyclonal antibody had dilution ratio of 1:250 compared with both the laboratories using EP41 from biocare had dilution of 1:50.

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

   It is not apparent in this run.

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

   Yes.

   Both laboratories using acidic pH (6.0 and 6.2) had poor 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?**

   No statistical difference was noted.

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

   Best performance was achieved with a combination of polyclonal antibody, HIER at alkaline pH and optimum retrieval time. Majority of the laboratories used RTU antibodies. At least 3 laboratories had significant background staining affecting interpretation. This could be because of polyclonal antibody being used. In that sense, monoclonal antibodies may be a better choice. However, one may consider dilution of primary antibody in the experimental setting to check on this aspect.

   One laboratory used clone UCHT1 where the company (Biogenex) clearly mentions intended use in frozen section material. Clone PS1 from the same company is intended for use in FFPE tissue. The laboratory should be careful in using antibody and study the clones prior to ordering.

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

    It is not possible to pinpoint poorly performing antibody.

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

    a. Poor clone and vendor selection
    b. HIER buffer at acidic pH

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

    Tonsil appears to be the best and easily available control material for this marker.

Recommended protocol for CD3 for RTU

Obtained in General Module, Run 2

Primary antibody

Clone	Rabbit polyclonal
Producer	Dako
Product no. (Lot no.)	IS503
Dilution	RTU
Diluent buffer and additive(s)	NA
Incubation time / temperature	30 min./RT

Epitope retrieval, HIER

Device	Manual
Buffer, pH	TBST, pH 9.2
Peak temperature and time	96 C. 20 minutes

Visualization system

Method	Polymer conjugate
Producer, product no.	Dako-Real Envision K5007
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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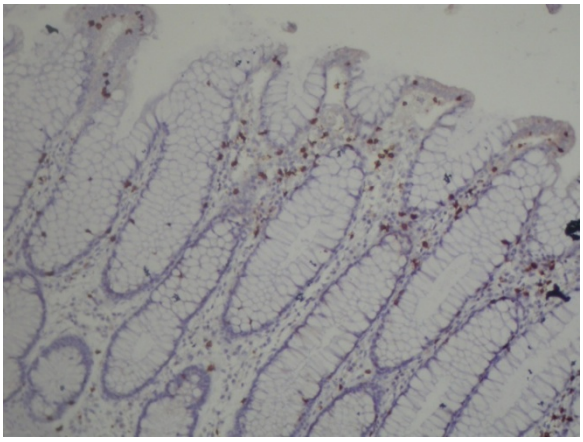

Fig.1: Optimum staining of colonic mucosal T-cells using polyclonal Dako RTU antibody IS503 with HIER at pH 9.2, 96 C for 20 min.

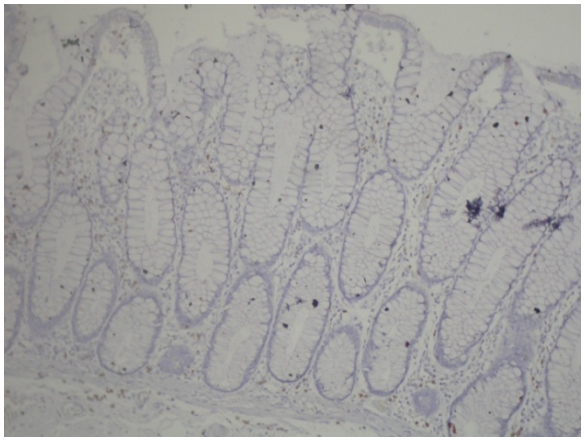

Fig.2: Suboptimal staining of colonic T-cells. Very few cells are stained. Clone EP41 was used in 1:50 dilution at retrieval buffer pH of 6.2.

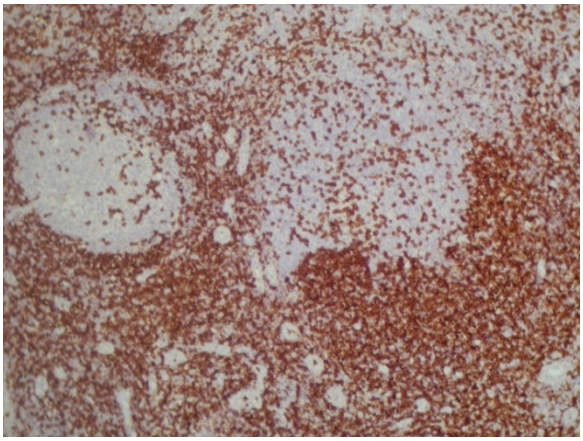

Fig.3: Optimum staining of tonsillar tissue using same protocol as Fig.1

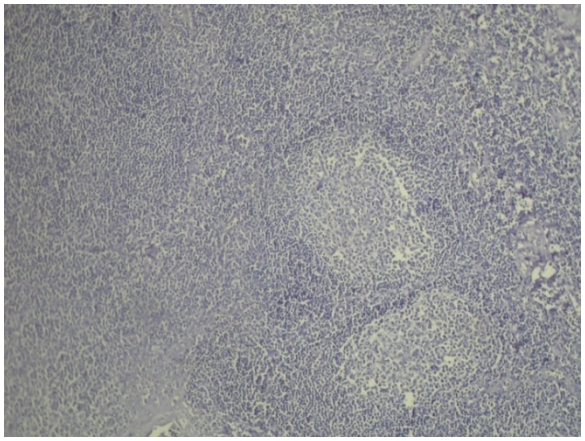

Fig.4: No staining at all. Clone UCHT1 was used that is intended for frozen section only.

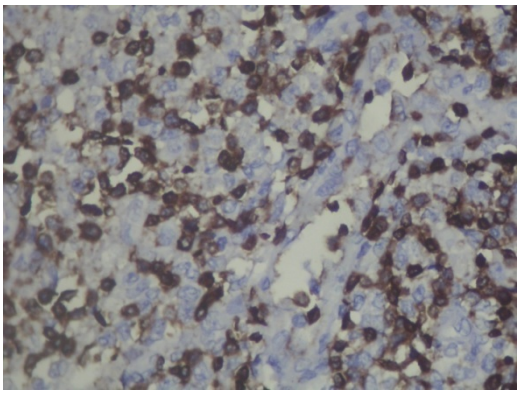

Fig.5: Optimum staining of PTCL cells using clone PS1 with HIER at alkaline pH

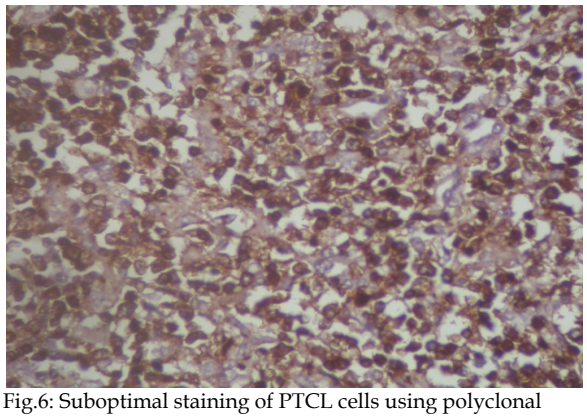

Fig.6: Suboptimal staining of PTCL cells using polyclonal Cell Marque antibody HIER at alkaline pH. Heavy background staining.