General module. Cycle 1, Run 2

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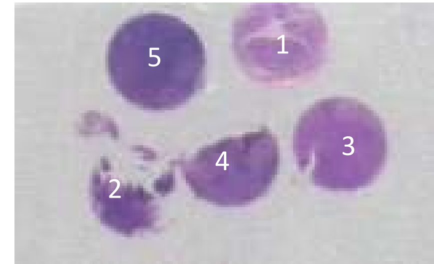
May 2015



The slides to be stained for CD20 comprised :

1. Appendix,
2. Tonsil,
3. DLBCL,
4. Follicular lymphoma, and
5. B-CLL

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

Criteria for assessing staining as optimal were:

<table>
<tr><th>Tissue</th><th>Reaction pattern</th></tr>
<tr><td>Appendix</td><td rowspan="2">A strong, predominantly membranous staining reaction of the mantle zone B-cells, the germinal centre B-cells and the interfollicular B-cells in the tonsil and the appendix</td></tr>
<tr><td>Tonsil</td></tr>
<tr><td>DLBCL</td><td>A strong membranous staining reaction of virtually all the neoplastic cells of the DLBCL</td></tr>
<tr><td>Follicular lymphoma</td><td>A moderate to strong membranous staining reaction of virtually all the neoplastic cells of the follicular lymphoma</td></tr>
<tr><td>B-CLL</td><td>A moderate to strong membranous staining reaction of virtually all the neoplastic cells of the B-CLL</td></tr>
</table>

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

Results:

The performance of the group was averaging 64% 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%	1
Borderline	>70%	0
Poor	</=70%	8

Antibody details and performance analysis based on critical parameter score:

RTU	Conc	Vendor	N	Clone	Optimum	Good	Borderline	Poor
12		Dako, IS604	6	L26	4 (66%)	X	X	2 (34%)
		Biogenex, AM238	2	L26	X	X	X	2 (100%)
		Biogenex, AM537	1	CD20/C3				1 (100%)
		CellMarque, 120M-87	1	L26	X	X	1 (100%)	X
		Leica, PA0906	1	MJ1	X	X	X	1 (100%)
		Thermo, MS-340-R7	1	L26	X	X	X	1 (100%)
	2	Dako, M0755	1	L26	1 (100%)	X	X	X
		Biocare CM004A	1	L26	X	X	X	1 (100%)

Comments:

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

   It is obvious from above results that clone L26 produced best results. However, the overall results in the run are not so promising. The major reason for that is inadequate staining of B-CLL cases. Cases of B-CLL inherently show poor staining with L26. One must validate antibody using this case for optimum performance. Majority of the laboratories validate antibodies on tonsil/appendix/lymph node tissue. In that case, criteria for optimum staining should be stringent and weak staining of any degree MUST NOT be entertained.

   Majority of the laboratories used RTU antibody. This prevents modification in dilution to offset suboptimum staining. We in our lab prefer concentrated antibody for this marker as we have found that the dilution needs to be changed relatively frequently for this marker.

2. Which vendor has the best performance?

In L26 group, Dako antibody was the best performing one.

3. What was better, RTU or Concentrated?

-

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

The best performing Dako antibody clone L26 had dilution ratio of 1:400.

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

Interestingly, 3 out of 4 labs using automated system had dewaxing temperature of more than 70 C (average 74 C). Only one laboratory had optimum staining (Ventana using 75 C) while the remaining two (Ventana and Leica) had poor staining. The laboratory having optimum staining used HIER at 100 C for 4 minutes compared with 20 min and 60 min in cases of poorly performing labs.

The remaining 1 lab in automated system Ventana Benchmark used dewaxing temperature of 60 C and had the best performance in the run.

Higher dewaxing temperature may not be the sole responsible factor for suboptimal staining. However, it appears to be important determinant in overall optimum staining.

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 Clone L26, HIER at alkaline pH and optimum retrieval time. Majority of the laboratories used RTU antibodies. This may not allow them to tweak dilution that becomes necessary at times, especially to detect low expressors of CD20 (e.g. B-CLL). Inability to stain cases of B-CLL was the major attribute for poor performance in this run.

10. Which antibody clones had poor performance?

Clones other than L26 had poor performance statistics in this run.

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

a. Poor clone selection
b. Poor vendor selection with proper clone
c. HIER buffer at acidic pH
d. Inability to tweak dilution as most used RTU antibody.

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

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

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Recommended protocol for CD20 for RTU

Obtained in General Module, Run 2

Primary antibody	
Clone	L26
Producer	Dako
Product no. (Lot no.)	M0755
Dilution	1:400
Diluent buffer and additive(s)	Dako antibody dilutent, S0809
Incubation time / temperature	30 min./RT

Epitope retrieval, HIER	
Device	Manual
Buffer, pH	Tris-EDTA buffer at pH 9.0
Peak temperature and time	95 C. 20 minutes in MWO

Visualization system	
Method	Polymer conjugate
Producer, product no.	Dako-Real Envision K5007
Incubation time / temperature	30 min./RT

Chromogen	
Type	DAB
Enhancement, type	Copper sulphate

Immunostainer	
Type	Manual

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

For images, turn the page:

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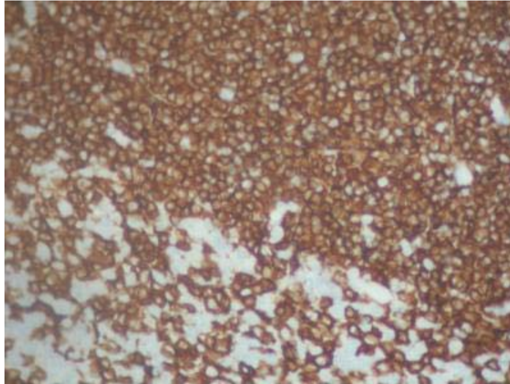

Fig.1: Optimum staining of lymphoid cells in tonsillar lymphoid tissue. Such staining will not miss staining of B-CLL case. Ventana Benchmark with Dako L26 clone.

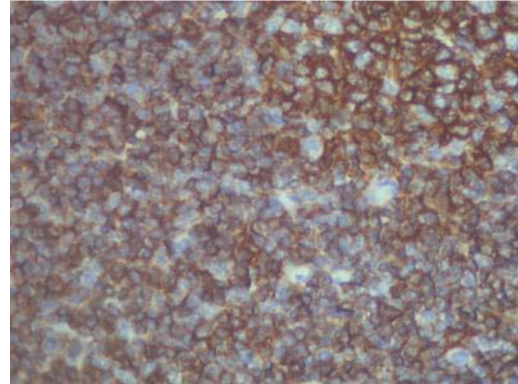

Fig.2: Suboptimal staining of same tissue. This would miss B-CLL case. Clone L26, Biogenex at alkaline pH.

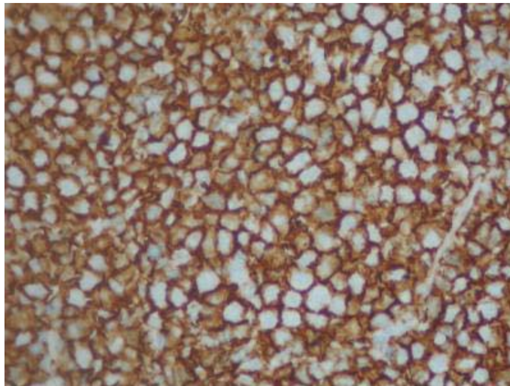

Fig.3: Crisp and intense staining of DLBCL case. Uses automated Ventana system with Dako L26 (not same lab as Fig.1)

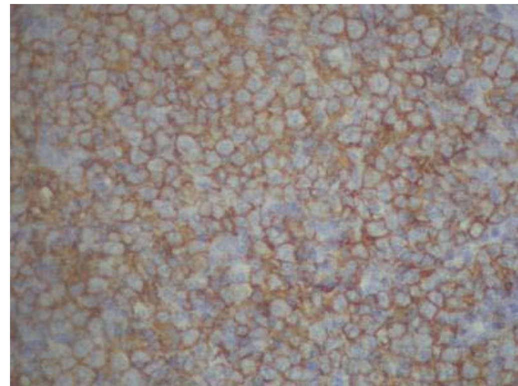

Fig.4: Suboptimal staining of DLBCL case. Uses Biocare L26 at acidic pH (6.0)

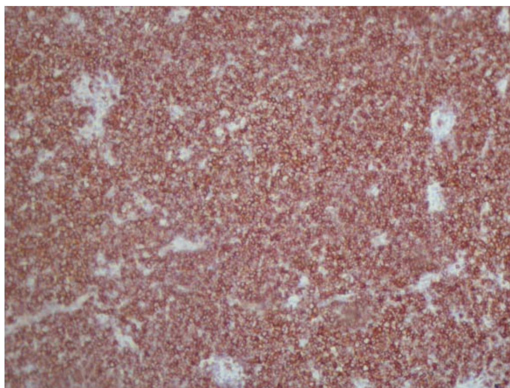

Fig.5: Optimum staining of B-CLL case. Dako L26. HIER at 96 C for 20 min in MWO.