General module. Cycle 1, Run 1

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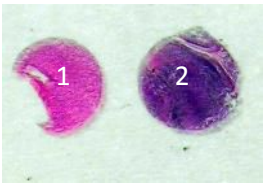
The slides to be stained for CD15 comprised :

1. Kidney, 2. Hodgkin lymphoma



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

Criteria for assessing staining as optimal were:

- A moderate to strong predominantly membranous staining reaction of the epithelial cells lining the
  renal proximal tubules
- A moderate to strong and distinct predominantly membranous staining reaction as well as dot-like (Golgi) staining reaction of the vast majority of Hodgkin and Reed-Sternberg cells in the two Hodgkin lymphomas. Strong cytoplasmic staining in neutrophil granules in all four specimens

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%	0
Borderline	>70%	3
Poor	</=70%	2

Antibody details and performance analysis:

RTU	Conc	Vendor	N	Clone	Optimal	Good	Borderline	Poor
7		Dako	5	Carb-3	2	0	2	1
		Thermo	1	AB-3				1
		Cellmarque	1	MMA	1			1
	1	Biocare	1	MMA+BY87			1	

This parameter saw less number of participants with optimal or good staining. There were more borderline and poor scores. RTU was distinctly more popular than concentrated ones. Optimal staining could be obtained only with Dako (2/5,) and Cell Marque antibody (1/1).

Clone MMA from Cell Marque yielded the best performance statistics. Carb-3 was another successful clone.

Clones AB3 and MMA+By87 **DID NOT** yield optimal or good results.

2 participants used expired antibodies; 1 yielded optimum score and the other one poor.

HIER was used as epitope retrival method by one and all.

The best performance statistics were achieved by:

those who used either Carb-3 or MMA clone, with HIER, using Tris-EDTA buffer at alkaline pH (9.0). The best two performers used MWO for heating. The heating ranged from 90 to 95 degrees and for 20 minutes to 40 minutes in those with better results. Typical incubation time of primary ranged from 30 minutes to 60 minutes.

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 4 laboratories had optimal or good staining performance *in critical parameter analysis*. Following parameters were central to optimal staining performance:

RTU antibodies (4/4):

All 4 used manual method.

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

The efficient heating time ranged from 90 to 95 degrees and for 20 to 30 minutes.

Primary antibody incubation time was 30 min in 2 and 60 min in other 2.

Secondary were all polymer based (Dako, Cell Marque and Biocare).

One lab used 'expired' primary antibody.

Concentrated antibodies (0/4):

Conclusions:

- Overall there was poor performance by the group for this marker. The insufficient staining was seen as too weak or negative staining of the expected cells. The participants were able to demonstrate good staining of granulocytes. Insufficient HIER (too short heating time or too short temperature) and less sensitive detection systems appear to be the cause. However, choice of primary antibody had more bearing on the final outcome.
- Clones MMA and Carb-3 are good clones.
- Clones AB3 and By87 did not yield good results.
- Demonstration of CD15 using three different mAbs for CD15 (BY87, MMA and Carb-3) has recently been tested thoroughly using in-house optimized protocols (Røge et al. Appl Immunnohistochem Mol Morphol 2014;22:449-458), showing that the mAb BY87 was inferior to the concentrated formats of Carb-3 and MMA. This result is in concordance with the results of this run.
- MWO heating gives better results.
- Alkaline pH of 9.0 has better performance.
- Kidney and tonsil are recommended as positive and negative **tissue controls** for CD15. Appendix with well-developed germinal centre could be an alternate. In the kidney the protocol must be calibrated to provide a distinct and strong predominantly membranous staining reaction in virtually all the epithelial cells of the proximal tubules. In tonsil/appendix, follicular dendritic cells of the germinal centres must show an at least weak but distinct predominantly membranous staining reaction (the proportion of follicular dendritic cells can vary from tonsil to tonsil). The neutrophil granulocytes will show a strong cytoplasmic staining reaction. All other cell types including B- and T cells must be negative. As a supplement to kidney and tonsil, especially in the technical calibration phase of the CD15 assay, it is recommended to verify the protocol on Hodgkin lymphomas, classical subtype.

Recommended protocol for CD15 for RTU

Obtained in General Module, run 1

Primary antibody

Clone	MMA
Producer	CELL MARQUE; CAT NO 115M-17
Product no. (Lot no.)	21080E
Dilution	RTU
Diluent buffer and additive(s)	NA
Incubation time / temperature	60 min./RT

Epitope retrieval, HIER

Device	Manual
Buffer, pH	Tris-EDTA based buffer, pH 9.0
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	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

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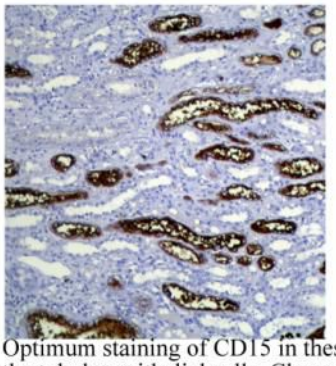
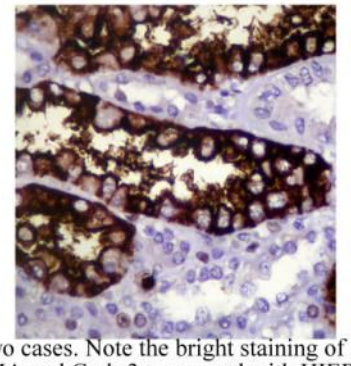
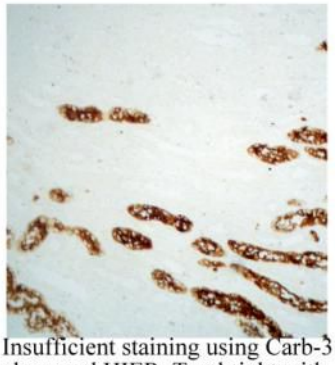
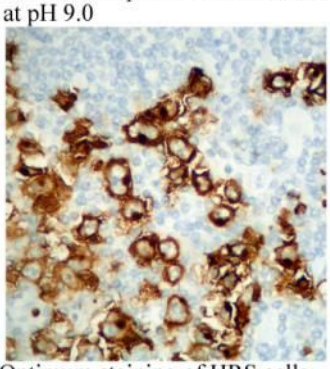




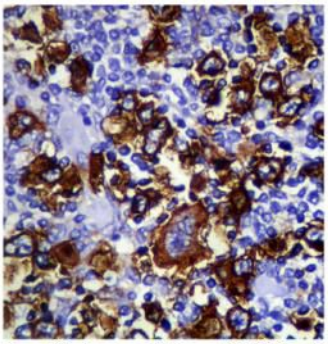
Optimum staining of CD15 in these two cases. Note the bright staining of the tubular epithelial cells. Clones MMA and Carb-3 were used with HIER at pH 9.0



Insufficient staining using Carb-3 clone and HIER. Too bright with poor counterstain.



Optimum staining of HRS cells using Carb-3 clone, HIER at 95 C for 30 min.



Insufficient staining of HRS cells. Note predominant cytoplasmic staining.

Insufficient staining using clone AB3. Too high temperature for dewaxing and HIER.