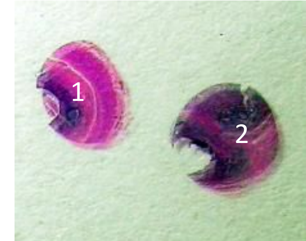
General module. Cycle 1, Run 1



The slides to be stained for CD30 comprised :

1. Appendix, 2. Hodgkin lymphoma

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

Criteria for assessing staining as optimal were:

- An at least weak to moderate and distinct membranous staining of the activated interfollicular and perifollicular B- & T-cells in the appendicular lymphoid collections **(This was found to be insufficient in trial runs as well and hence not counted for the assessment)**
- An at least weak to moderate, predominantly membranous and dot-like cytoplasmic staining of the majority of the Hodgkin cells

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%	1
Good	≥80%	3
Borderline	≥70%	0
Poor	<70%	4

Antibody details and performance analysis:

RTU	Conc	Vendor	N	Clone	Optimal	Good	Borderline	Poor
6		Dako	4	Ber H2		1		3
		Cellmarque	1			1		
		PathnSitu	1	EP154				1
	2	Dako	1	Ber H2		1		
		Biocare	1	CON6D/B5	1			

The most common cause of insufficient staining was predominant cytoplasmic staining rather than 'dot-like' staining that should be seen in HRS cells. This could have been because of either too high titer of the antibody (that could not have been avoided as majority used RTUs) and/or inadequate washing in buffer.

(It is often observed that the labs employ best detection systems and use RTU antibodies. The RTUs are tittered to work in the least sensitive detection systems. In that scenario, it becomes difficult to avoid non-specific and background staining. The companies do not recommend diluting the RTU primary antibodies further. However, I do dilute them in several instances as in my personal experience this has worked well (when I have encountered above issues))

Inadequate HIER was another cause of weak staining.

Conclusions:

- The best result was obtained using the concentrated antibody clone CON6D/B5 from Biocare.
- Ber H2 remained the most popular clone.
- Clone EP154 is a newer clone. The result in this run was not found to be optimum/good. However, it appeared to be a problem with dewaxing temperature, HIER temperature and pre-analytic variables in that case.
- Both the labs using concentrated antibodies had best performance in the group. This is probably due to they being able to adjust the best suitable dilution. The range of dilution has been 1:100 to 1:250.
- All the laboratories used HIER as retrieval method using either MWO or pressurized systems. Inferior results were obtained when HIER temperature was low or high in relation to the time.

- Tonsil/appendix and Hodgkin lymphoma are supposed to be the best control materials for CD30. However, in this run we could not assess appendicular lymphoid tissue as we found that tissue trailing in this run. This was confirmed in the subsequent trial runs as well.

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 3 laboratories had optimal/good staining performance in critical parameter analysis. Following parameters were central to optimal staining performance:

RTU antibodies (1/3):

The lab used manual method. The clone used has been Ber-H2 from Dako.

Optimal staining results obtained using HIER as retrieval method in Tris-EDTA buffer at pH 9.0.

The heating time has been 95 degrees C for 30 minutes at 0.1 psi. Biocare decloaking chamber has been used.

Primary antibody incubation time was 60 min.

Secondary was polymer based, MACH1, BIOCARE MEDICAL, CAT No: M1U539L10, Batch No: 080514, expiry : 2015/11, with incubation time of 30 minutes.

Concentrated antibodies (2/3):

Both used manual method. The clones used were Ber H2 from Dako in 1:100 dilution and CON6D/B5 from Biocare in 1:250 dilution.

HIER as retrieval method in Tris-EDTA buffer at pH 9.0

The heating time has been 95 degree for 30 minutes and 40 minutes for them.

Primary antibody incubation time was 30 min and 40 minutes.

Secondary used were Dako Envision, polymer based with incubation time of 30 minutes and MACH1 Universal HRP Polymer Kit , Biocare Medicals, M1U539L10, 062014, 2014/March, Exp: 10/2015 for 40 minutes.

One of them used primary antibody ‘expired’ in year 2011!

Recommended protocol for CD30 for RTU

Obtained in General Module, run 1

Primary antibody

Clone	Ber H2

Producer	Dako cat no. IS 602
Product no. (Lot no.)	20005421
Dilution	RTU
Diluent buffer and additive(s)	NA
Incubation time / temperature	60 min./RT

Epitope retrieval, HIER

Device	Decloaking chamber, Biocare
Buffer, pH	Tris-EDTA buffer, pH 9
Warm-up / heating max / resting time	95 degree for 30 minutes, 0.1 psi

Visualization system

Method	2-step polymer conjugate
Producer, product no.	MACH1, BIOCARE MEDICAL, CAT No: M1U539L10, Batch No: 080514
Incubation time / temperature	30 min./RT

Chromogen

Type	DAB
Enhancement, type	Nil

Immunostainer

Type	Manual

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

Steps in brief:

1)Fixation 2) Deparffinization 3) rehydration 15 mins(5x3) 4) Wash under running water 10 mins 5) Antigen retrieval 30 mins 6) Bring to RT 10 mins 7) Rinse in deionized water 8)Rinse with TBS 9) 3% H2O2 10 mins 10) Rinse twice in TBS 11) Background quencher 10 mins (12) Rinse in TBS (13) Primary antibody incubation 60 mins (14) Rinse with TBS twice 15) Probe incubation 10 mins 16) Rinse with TBS(17)HRP 30 mins 18)Rinse with TBS thrice 19) DAB chromogen 5 mins (20) Wash with deionized water (21)Counterstain with haematoxylin 5 mins 22)Clear with Xylene and mount

Recommended protocol for CD30 for concentrated antibody

Obtained in General Module, run 1

Primary antibody

Clone	CON6D/B5
Producer	Biocare CM346A

General module. Cycle 1, Run 1

www.qcmark.org

Product no. (Lot no.)	080113
Dilution	1:250
Diluent buffer and additive(s)	Tris-based solution called Renoir Red PH 6.0, Biocare , PD904H
Incubation time / temperature	40 min./RT
Epitope retrieval, HIER	
Device	Decloaking chamber
Buffer, pH	Tris-EDTA buffer, pH 9
Warm-up / heating max / resting time	95 degree for 40 min
Visualization system	
Method	Polymer conjugate
Producer, product no.	MACH1 Universal HRP Polymer Kit , Biocare Medicals, M1U539L10, 062014, 2014/March, Exp: 10/2015
Incubation time / temperature	30 min./RT
Chromogen	
Type	DAB
Enhancement, type	
Immunostainer	
Type	Manual

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

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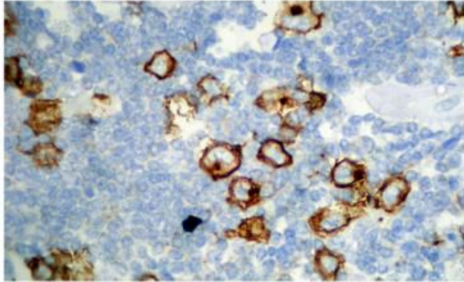
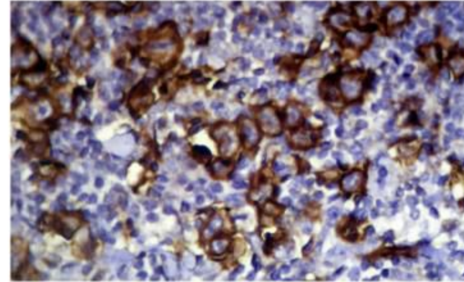
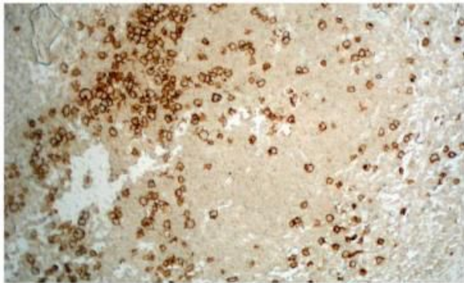


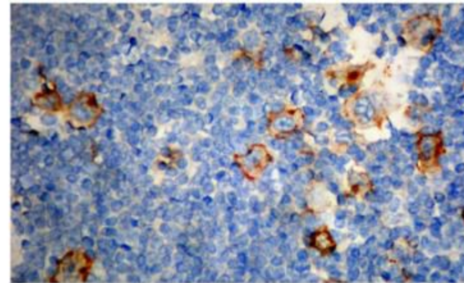
Optimum staining of HRS cells using clone CON6D/B5 in 1:250 dilution and HIER. Note the positivity of membrane and golgi region.



Insufficient staining of HRS cells. Though the cells are stained, they are predominantly cyto-plasmic



Suboptimal staining of HL case. Though the tumor cells are stained, the background is too heavy. RTU was used



Insufficient staining for CD30. The cells are too weakly stained. EP154 clone, RTU was used.