Summary Report – Run 122 CD20

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Overview

CD20 is a B-cell marker of clinical significance for the use of rituximab, an anti-CD20 antibody, in the treatment of lymphomas. For Run 122 CD20 a multi-tissue section containing appendix, pancreas, tonsil, and liver, as well as a 28-core lymphoma tissue microarray were included. Participants were asked to assess only the multi-tissue control section. CPQA provided assessment of the 28-core tissue microarray for which this summary is based on, using the following score system:

- **Positive (P) –** Any definitive membranous/cytoplasmic staining
- **Negative (N) –** No staining
- **Unsatisfactory (U) –** Tissue core not suitable for assessment e.g. no tumour cells, no core, etc.

The CPQA appreciates that the score system used by laboratories can vary by institution and simply used the above to establish consistent reporting.

Results

Cores 7, 15 and 24 had weak expression of CD20. Overall, CD20 staining was excellent. Participant-specific feedback is provided below:

Lab ID	IHC Status*	Comment
101	Adequate	Weak staining
102	Optimal	
103	Optimal	
112	Optimal	
114	Adequate	Weak staining
120	Optimal	
129	Optimal	
147	Optimal	
149	Adequate	Weak staining
151	Optimal	
198	Adequate	Weak staining
207	Optimal	
230	Optimal	

*based on CPQA assessor consensus

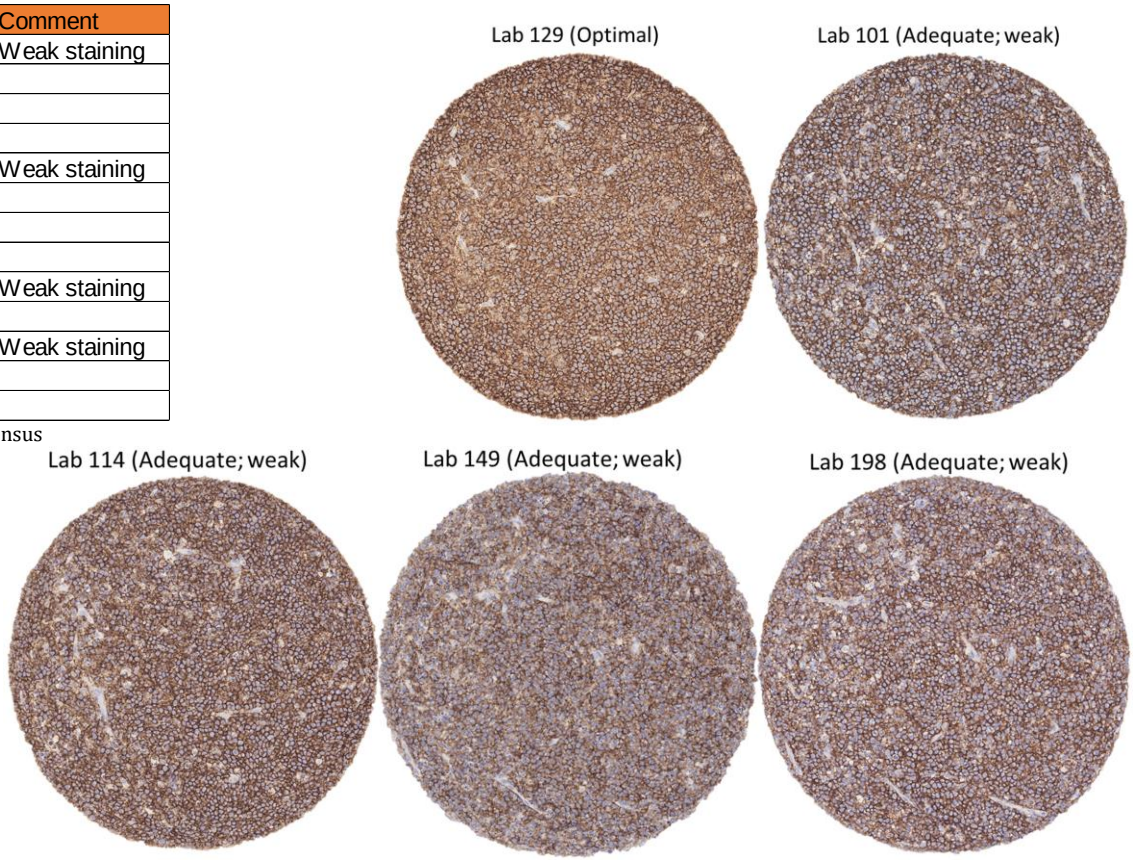

Figure 1. Representative images of the qualitative variability of strong positive CD20 staining.

Garrattogram after CPQA assessment:

Lab ID	101	102	103	112	114	120	129	147	149	151	198	207	230	R1
1	P	P	P	P	P	P	P	P	P	P	P	P	P	P
2	P	P	P	P	P	P	P	P	P	P	P	P	P	P
3	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	P	P	P	P	P	P	P	P	P	P	P	P	P	P
5	U	U	U	U	U	U	U	U	U	U	U	U	U	P
6	P	P	P	P	P	P	P	P	P	P	P	P	P	P
7	P	P	P	P	P	P	P	P	N	P	N	P	P	P
8	P	P	P	P	P	P	P	P	P	P	P	P	P	P
9	P	P	P	P	P	P	P	P	P	P	P	P	P	P
10	P	P	P	P	P	P	P	P	P	P	P	P	P	P
11	P	P	P	P	P	P	P	P	P	P	P	P	P	P
12	N	N	N	N	N	N	N	N	N	N	N	N	N	N
13	P	P	P	P	P	P	P	P	P	P	U	U	P	P
14	P	P	P	P	P	P	P	P	P	P	P	P	P	P
15	P	P	P	P	P	P	P	P	N	P	N	P	P	P
16	P	P	P	P	P	P	P	P	P	P	P	P	P	P
17	N	N	N	N	N	N	N	N	N	N	N	N	N	N
18	U	U	U	U	U	U	U	U	U	U	U	U	U	P
19	P	P	P	P	P	P	P	P	P	P	P	P	P	P
20	P	P	P	P	P	P	P	P	P	P	P	P	P	P
21	P	P	P	P	P	P	P	P	P	P	P	P	P	P
22	P	P	P	P	P	P	P	P	P	P	P	P	P	P
23	N	N	N	N	N	N	N	N	N	N	N	N	N	N
24	P	P	P	P	P	P	P	P	N	P	N	P	P	P
25	P	P	P	P	P	P	P	P	P	P	P	P	P	P
26	P	P	P	P	P	P	P	P	P	P	P	P	P	P
27	P	P	P	P	P	P	P	P	P	P	P	P	P	P
28	P	P	P	P	P	P	P	P	P	P	P	P	P	P

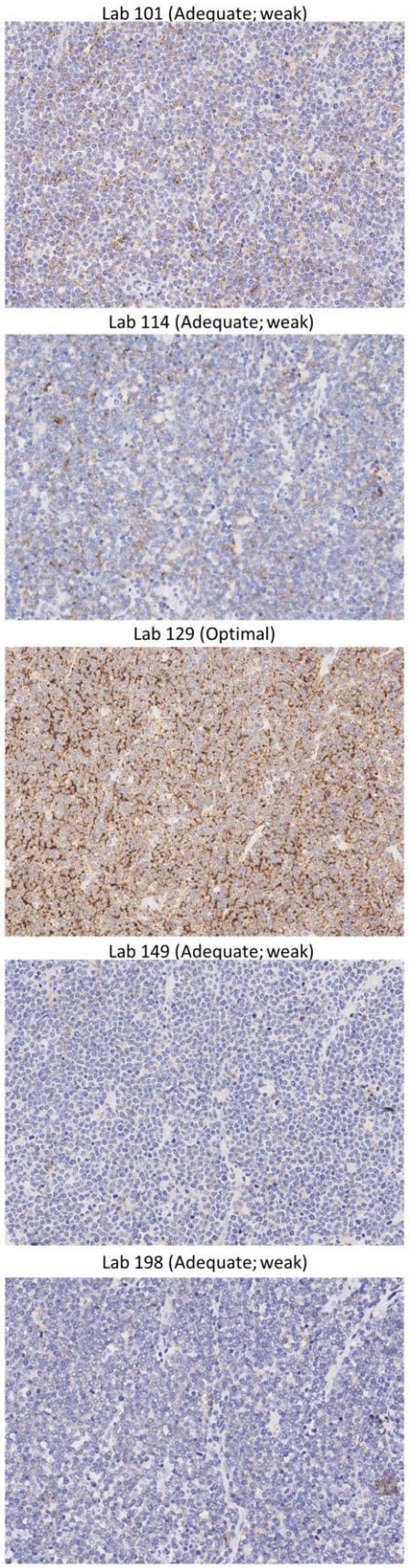

Figure 2. Representative images of the qualitative variability CD20 staining in a weak positive case.

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Supplementary Table 1 summarizes the reported staining protocols, which can be referred to during validation or optimization of a staining protocol. Supplementary Table 2 summarizes descriptive statistics based on CPQA assessment. Quality control methodologies of immunohistochemical assessment are evolving, and numeric results should be interpreted with this reservation. Supplementary Table 3 provides the definitions of IHC Status and recommended participant action. Your regular participation in CPQA is greatly appreciated and we look forward to continuing to work with you and the Canadian Association of Pathologists – Association canadienne des pathologistes.

Scanned images in this report were acquired using a NanoZoomer SQ that has been graciously loaned to the CPQA-AQCP by Quorum Technologies and Hamamatsu.

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Table S1. Reported CD20 staining protocols.

Lab ID	Platform/Instrument	LDT or IHC Kit	Ag Retrieval Method	Time for Ag Retrieval (min)	Ab Clone	Ab Dilution	Ab Supplier/ Vendor	Ab Lot No.	Time for Ab Incubation (min)	Detection System	Amplification (Y/N)	CuSO4, etc. (Y/N)	Chromogen
101	DAKO OMNIS	LDT	EnVision FLEX TRS HIGH pH	30 MIN	L26	RTU	DAKO	20075401	10 MIN	DAKO Envision FLEX	N	N	DAB
102	Dako Autostainer 48 Link	LDT	Dako retrieval solution 9.0	20	L26	1:400	Dako	20069530	30" RT	Dako Envision Flex	N	yes CuSO4	Dab+
103	BENCHMARK ULTRA	LDT	CC1	20 mins	L26	Pre	Ventana/Roche	G02279	32	Ultraview	N	Y copper	DAB
112	BOND III	LDT	BOND ER1 pH6.0	20 minutes	L26	RTU	Leica	67591	15 minutes @ RT	BOND Polymer Refine Detection	no	no	DAB
114	Dako Omnis	LDT	Envision Flex TRS, low pH	30	L26	1:100	Dako	20062092	10	Envision Flex DAKO Omnis	N	N	Envision FLEX DAB+
120	Autostainer Link48	Kit	HIER Waterbath	20	L26	RTU	Dako	20075398	20	Envision Flex+	N	N	DAB
129	BOND III	LDT	ER 2- high pH retrieval	20	L26	1:400	DAKO	20019743	15	bond refine detection kit	N	N	DAB
147	LEICA BOND 3	LDT	ER 2 PH 8	20	L 26	1000	DAKO	20051534	15	LEICA REFINE KIT	N	N	DAB
149	OMNIS Agilent/Dako	LDT	high pH 97 C	30	L26	RTU	Agilent/Dako	20066815	20	EnVision Flex	No	No	DAB
151	BOND111	LDT	HIER 2	20MIN	L26	1:1200	DAKO	20028627	15 MIN	BOND REFINE	N	N	DAB
198	Dako Omnis	LDT	HIER	30	L26	RTU	Agilent	20075401	20	Envision Flex	N	N	DAB
207	Benchmark Ultra	LDT	online	36	L26	prediluted	Vantana	G02279	32	Ultra view DAB detection system	N	Y	DAB
230	Benchmark Ultra	LDT	HIER	24	L26	predilute	Roche Diagnostics	G02279	24	OPTIVIEW	N	N	DAB

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Table S2. Descriptive statistics based on CPQA assessment.

Lab ID	Total n	% scorable	Pairwise complete observations	Concordance with reference (%)	Sensitivity	Specificity	Cohen's kappa
101	28	92.86	26	26/26 (100%)	1	1	1
102	28	92.86	26	26/26 (100%)	1	1	1
103	28	92.86	26	26/26 (100%)	1	1	1
112	28	92.86	26	26/26 (100%)	1	1	1
114	28	92.86	26	26/26 (100%)	1	1	1
120	28	92.86	26	26/26 (100%)	1	1	1
129	28	92.86	26	26/26 (100%)	1	1	1
147	28	92.86	26	26/26 (100%)	1	1	1
149	28	92.86	26	23/26 (88%)	0.86	1	0.66
151	28	92.86	26	26/26 (100%)	1	1	1
198	28	89.29	25	22/25 (88%)	0.86	1	0.66
207	28	89.29	25	25/25 (100%)	1	1	1
230	28	92.86	26	26/26 (100%)	1	1	1

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Table S3. Proficiency Testing Definitions of IHC Status.

IHC Status	Definition	Proficiency Testing Performance
Optimal	All expected targets are identified appropriately and demonstrate the expected staining intensity. Absence of non-specific staining (no background staining).	**PASS**
Adequate	All targets are identified, but intensity of staining is weaker than optimal or there is false-positive staining which does not interfere with interpretation.	**PASS**
Sub-optimal	None or only some targets are identified OR all targets are identified, but false-positive staining may interfere with interpretation.	**PASS, Conditionally[1]**
Failed	The staining was considered to be of such poor quality that accurate readout of the test is unlikely or impossible.	**FAIL[2]**
Unsatisfactory	Technical issue (e.g. unsuitable antibody selection, etc.)	**N/A**

1 – A one-time sub-optimal performance qualifies for a "Pass" result. Two successive "sub-optimal" results will be designated as a "Fail".

1,2 – Please contact the CPQA for assistance and, if necessary, inform your regional regulatory body as per the terms of your laboratory's accreditation provider.