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Standardization of CD30 immunohistochemistry staining among three automated immunostaining platforms

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CD30 を標的とする抗体薬物複合体を用いたリンパ腫の治療には、免疫組織化学による CD30 発現の同定が不可欠である。しかし、CD30 染色の標準化されたプロトコールはこれまでなかった。本研究では、3 つの一般的な自動免疫染色プラットフォーム｛Bond III（B III）、Dako Omnis（DO）、Ventana BenchMark ULTRA（VBMU）｝を用いて CD30 の染色性について比較検討した。CD30 の一次抗体である Ber-H2 クローンは、B III と DO では 50～400 倍に希釈され、VBMU では希釈済の抗体を用いた。DO では、リンカーを用いたエンハンスメントステップが導入された。まず、6 症例を染色して、各プラットフォームについていくつかの希釈候補(B III, 200x, 400x, DO 100x, 200x)を選択した。次に、これらの候補条件を用いて末梢性 T 細胞リンパ腫（PTCL）60 症例(ATLL, 30 例; AITL, 10 例 ALCL, 5 例 ; PTCL-NOS, 15 例)で確認した。CD30 発現の一致率は、未分化大細胞リンパ腫を除き、カットオフ値や抗体希釈度によってプラットフォーム間で異なっていた。BⅢでは 400 倍希釈抗体、DO では 100 倍希釈抗体を用いた場合、カットオフ値を 1%、10%とした場合の「陽性」「陰性」の判定における 3 プラットフォーム間の一致率は、それぞれ 100%、97%であった。本研究は、プロトコールを調整することにより、異なるプラットフォーム間で PTCL の CD30 染色を均等にすることが可能であることを示した。

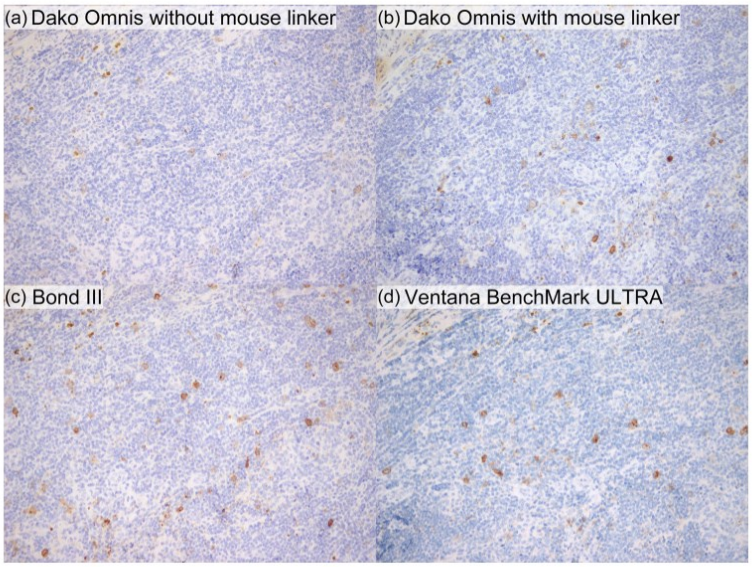

FIGURE 1 CD30 immunohistochemistry staining in tonsilitis using clone Ber-H2 (60x diluted, 3.13 μg/mL) by four different protocols. (a) Dako Omnis without a mouse linker; (b) Dako Omnis with a mouse linker; (c) Bond III; and (d) Ventana BenchMark ULTRA.

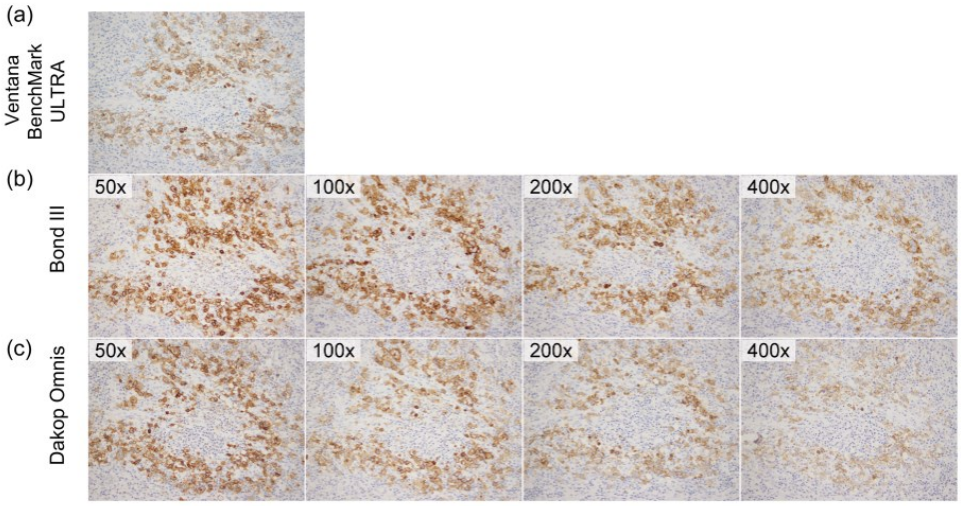

FIGURE 2 CD30 immunohistochemistry staining in classic Hodgkin lymphoma by different platforms and primary antibody concentrations. (a) Ventana BenchMark ULTRA using prediluted antibody; (b) Bond III using concentrated antibody diluted 50x, 100x, 200x and 400x; and (c) Dako Omnis using concentrated antibody diluted 50x, 100x, 200x and 400x.

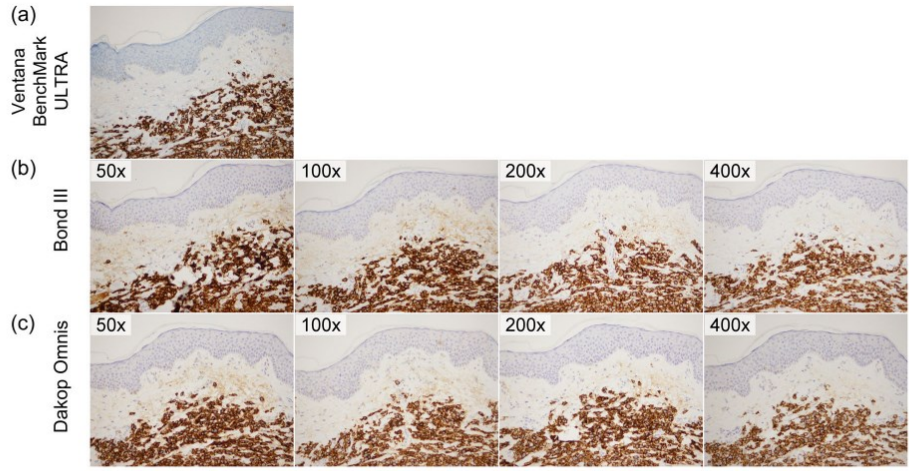

FIGURE 3 CD30 immunohistochemistry staining in anaplastic large cell lymphoma by different platforms and primary antibody concentrations. (a) Ventana BenchMark ULTRA using prediluted antibody; (b) Bond III using concentrated antibody diluted 50x, 100x, 200x and 400x; and (c) Dako Omnis using concentrated antibody diluted 50x, 100x, 200x and 400x.

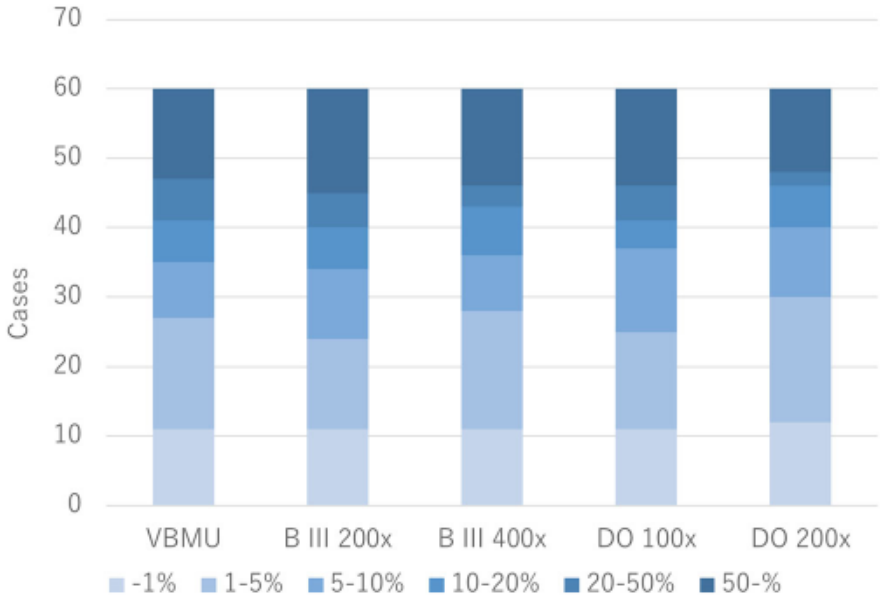

FIGURE 4 Number of cases with each peripheral T-cell lymphoma according to CD30-positive rate. Cases were classified into six levels (<1%, 1≤ and <5%, 5≤ and <10%, 10≤ and <20%, 20≤ and <50%, ≥50%) of CD30 positivity by four different protocols.

TABLE 1 Number and percentage of concordant cases of CD30 expression among three platforms depending on primary antibody concentration.

	VBMU vs B III 200x vs DO 100x	VBMU vs B III 200x vs DO 200x	VBMU vs B III 400x vs DO 100x	VBMU vs B III 400x vs DO 200x
ATLL	25/30 (83%)	19/30 (63%)	26/30 (87%)	21/30 (70%)
PTCL-NOS	13/15 (87%)	11/15 (73%)	12/15 (80%)	11/15 (73%)
AITL	8/10 (80%)	6/10 (60%)	8/10 (80%)	7/10 (70%)
ALCL	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)
Total	51/60 (85%)	41/60 (68%)	51/60 (85%)	45/60 (73%)

Abbreviations: AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ATLL, adult T-cell leukemia/lymphoma; B III 200x, Bond III with 200-fold diluted primary antibody; B III 400x, Bond III with 400-fold diluted primary antibody; DO 200x, Dako Omnis with 200-fold diluted primary antibody; DO 400x, Dako Omnis with 400-fold diluted primary antibody; PTCL-NOS, peripheral T-cell lymphoma, not otherwise specified; VBMU, Ventana BenchMark ULTRA.

Table 2. Number of discordant cases for CD30 expression level across each protocol, compared with Ventana BenchMark ULTRA

		B III 200x	B III 400x	DO 100x	DO 200x
CD30 positivity rate in VBMU	−1%	0/11	0/11	0/11	0/11
	1–5%	3/16 (3,0)	0/16	2/16 (2,0)	2/16 (1,1)
	5–10%	0/8	1/8 (0,1)	0/8	4/8 (0,4)
	10–20%	1/6 (1,0)	1/6 (0,1)	2/6 (0,2)	4/6 (0,4)
	20–50%	2/6 (2,0)	3/6 (1,2)	1/6 (1,0)	5/6 (0,5)
	50–%	0/13	0/13	0/13	1/13 (0,1)

Note: The figures in parentheses refer to the number of cases with a higher or lower CD30 positivity rate in each protocol than in VBMU, in order from the left.

Abbreviations: B III 200x, Bond III with 200-fold diluted primary antibody; B III 400x, Bond III with 400-fold diluted primary antibody; DO 200x, Dako Omnis with 200-fold diluted primary antibody; DO 400x, Dako Omnis with 400-fold diluted primary antibody; VBMU, Ventana BenchMark ULTRA.

Table 3. Positive and negative concordance rate of CD30 expression among the three platforms depending on primary antibody concentration across each cutoff.

		VBMU vs B III 200x vs DO 100x	VBMU vs B III 200x vs DO 200x	VBMU vs B III 400x vs DO 100x	VBMU vs B III 400x vs DO 200x
Cutoff	1%	100% (49, 49, 49)	98% (49, 49, 48)	100% (49, 49, 49)	98% (49, 49, 48)
	5%	93% (33, 36, 35)	88% (33, 36, 30)	95% (33, 32, 35)	92% (33, 32, 30)
	10%	95% (25, 26, 23)	90% (25, 26, 20)	97% (25, 24, 23)	92% (25, 24, 20)
	20%	98% (19, 20, 19)	90% (19, 20, 14)	97% (19, 17, 19)	92% (19, 17, 14)
	50%	97% (13, 15, 14)	95% (13, 15, 12)	97% (13, 14, 14)	97% (13, 14, 12)

Note: The figures in parentheses refer to the number of positive cases in VBMU, Bond III and Dako Omnis, in order from the left.

Abbreviations: B III 200x, Bond III with 200-fold diluted primary antibody; B III 400x, Bond III with 400-fold diluted primary antibody; DO 200x, Dako Omnis with 200-fold diluted primary antibody; DO 400x, Dako Omnis with 400-fold diluted primary antibody; VBMU, Ventana BenchMark ULTRA.