

Table 1: Pairwise results of current and new Her2Dual ISH Test

		Cytotech	Case ID	IHC Concurrent	HER2/CEP17 current	HER2/CEP17 new	CEP17 signal current	CEP17 signal new	HER2 signal current	HER2 signal new	DISH Group current	DISH Group new	comments (current)	comments (new)
Breast Cases														
Libby	JL	OSW20-2675 A1	3+	3.5	3.5	2.4	2.7	8.3	9.4	1	1	None	Signals appear clearer and easier to enumerate.	
Libby	KB	VS20-04602 A1	1+	1.0	1.0	2.0	2.0	2.1	2.1	5	5	Old stain.	New stain - signals appear clearer.	
Libby	KB	VS20-04566 A1	1+	1.0	1.0	2.2	2.3	2.1	2.2	5	5	Old stain	New stain - background much clearer than with old stain.	
Libby	JL	AS20-02331 A4	0	0.9	1.0	2.1	2.3	2.0	2.2	5	5	None	The signals appear to be cleaner, with more distinct borders.	
Libby	NP	OC20-01323 A1	1+	0.7	0.8	2.5	2.5	1.8	1.9	5	5	None	Signals are more pronounced - stronger staining.	
Libby	JL	VS20-04603 A1	3+	3.6	–	2.7	–	9.8	–	1	–	None	zHer2dual ISH failed due to circumferential staining.	
Libby	KB	VS20-04551 B4	–	–	1.2	–	2.0	–	2.3	–	–	Attempted but failed. No further testing for Her-2/neu will be performed unless specifically requested clinically.	New stain. Regular ISH failed.	

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Libby	NP	RS20-03964 A1	2+	0.7	0.8	2.1	2.2	1.5	1.8	5	5	None.	Marked improvement of HER2 stain - barely visible on original stain.
Libby	NP	AS20-02368 A1	2+	0.9	1.1	3.2	3.0	2.8	3.1	5	5	None	HER2 and Chr 17 signals stain much stronger - more easily identified.
Libby	NP	AS20-02368 B1	2+	0.9	1.1	3.2	2.9	3.0	3.1	5	5	–	Stronger staining of HER2 signals.
Libby	JL	VS20-04666 A1	2+	0.9	1.0	4.0	4.5	3.8	4.5	5	4	None	None
Libby	JL	VS20-04689 A1	1+	1.0	1.0	2.0	2.0	2.0	2.0	5	5	None	Background looks cleaner, signals appear a bit larger and more distinct.
Libby	KB	RS20-03964 A1	2+	0.7	0.8	2.1	2.2	1.5	1.8	5	5	Old Stain	New stain - clear signals and background.
Pizzi	NP	VS20-04737 A1	2+	1.0	1.1	2.6	2.6	2.7	2.9	5	5	None	Stains look very similar
Pizzi	NP	BFS20-0647 A1	1+	0.7	0.7	2.1	2.2	1.5	1.6	5	5	None	HER 2 signals much more visible with new stain.
Libby	KB	AS20-02418 A1	2+	1.1	1.1	2.1	2.2	2.3	2.4	5	5	Old stain - notable background dust	New stain - signals clear, much less background dust

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Libby	NP	AS20-02419 A1	2+	0.9	0.9	2.2	2.1	1.9	1.9	5	5	None	Stains look similar
Libby	KB	VS20-04710 A1	3+	4.7	5.0	1.9	2.0	8.9	10.0	1	1	Old stain	New stain - much clearer signals
Libby	KB	OS20-20293 A3	2+	2.1	2.4	2.2	2.6	4.7	6.1	1	1	Old stain	New stain - much better than old stain, signals are very clear.
Libby	JL	MS20-01672 C4	–	1.0	1.0	2.4	2.4	2.5	2.5	5	5	None	None
Libby	JL	MS20-01830 E9	0	1.0	1.0	2.2	2.4	2.2	2.5	5	5	None	None
Libby	NP	VS20-04739 C3	3+	1.9	1.9	2.9	2.9	5.5	5.3	4	4	None	Her2 signals much more defined
Libby	JL	AS20-02430 A1	1+	1.0	1.0	2.0	2.0	2.0	2.0	5	5	None	None
Libby	JL	OS20-20723 A2	2+	1.8	1.8	2.9	2.8	5.2	5.0	4	4	None	None
Libby	JL	MS20-01866 A2	0	0.8	0.8	2.0	2.0	1.5	1.7	5	5	Her2 signals are very faint, not present in some cells.	None
Libby	JL	OSW20-2800 A1	1+	1.0	1.0	2.0	2.0	2.0	2.0	5	5	None	None
Libby	JL	HS20-00055 A8	1+	1.0	1.0	2.2	2.2	2.3	2.3	5	5	None	None
Pizzi	KB	VS20-04842 A1	1+	1.0	1.0	2.1	2.0	2.0	2.0	5	5	Old Stain	New stain - clear signals.

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Pizzi	KB	BFS20-0659 A2	1+	1.0	1.0	2.1	2.0	2.0	2.0	5	5	Old stain	New stain - signals much clearer than old stain
Pizzi	KB	VS20-04854 A1	1+	1.0	1.0	2.0	2.0	2.0	2.1	5	5	Old stain	New stain - red signals appear clearer than old stain.
Andersen	KB	NS20-03790 B1	–	–	1.2	–	2.0	–	2.3	–	–	Her2DISH failed	New stain - regular slide failed.
Pizzi	JL	MS20-01914 B1	2+	1.0	1.0	2.4	2.4	2.4	2.4	5	5	None	Signals are slightly larger and more distinct with the new stain.
Pizzi	JL	MS20-01915 A1	0	1.0	1.0	2.0	2.0	2.0	2.0	5	5	None	None
Pizzi	JL	VS20-04878 A2	3+	5.6	5.6	2.3	2.3	12.6	12.9	1	1	None	Her2 signals are easier to enumerate with the new stain.
Andersen	NP	AS20-02492 A1	3+	6.2	6.1	2.3	2.7	14.1	16.1	1	1	Her2 counts are estimates due to crowded, overlapping signals.	Her2 signals very difficult to differentiate in new stain - larger, merged.
Andersen	NP	MS20-01920 A1	2+	0.9	0.9	2.4	2.5	2.2	2.3	5	5	None	Her2 signals appear larger, more distinct with new stain.
Pizzi	JL	RS20-03989 B2	–	–	1.1	–	2.5	–	2.9	–	–	Attempted but failed.	None

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Pizzi	NP	MS20-01818 A1	–	–	0.9	–	2.0	–	1.8	–	–	Attempted but failed.	Original failed. Had to search for individual countable cells, but Her2 and Chr 17 stain very strong.
Pizzi	JL	MS20-01808 B6	–	–	1.0	–	2.7	–	2.7	–	–	Attempted but failed.	None
Andersen	JL	VS20-04961 A1	2+	1.1	1.2	3.0	2.7	3.1	3.2	5	5	None	New stain is easier to enumerate, signals are slightly larger and more distinct.
Andersen	JL	VC20-00721 A1	2+	1.0	1.0	3.6	3.6	3.5	3.7	5	5	None	New stain is easier to enumerate, signals are more distinct.
Andersen	NP	SS20-00501 A1	1+	0.8	0.8	2.0	2.0	1.6	1.7	5	5	None	Her2 signals much more crisp, clearly defined.
Andersen	NP	MS20-01936 A1	1+	0.9	0.9	2.1	2.1	1.8	1.9	5	5	None	Her2 signal more clearly defined, bolder stain.
Andersen	NP	AC20-00173 A1	2+	1.2	1.4	3.0	2.7	3.5	3.8	5	5	None	Several more Her2 signals visible - stronger staining.
Andersen	KB	VS20-05009 A1	1+	1.0	1.1	2.1	2.0	2.1	2.1	5	5	Old Stain	New stain - nice clear signals, no background dust.

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Andersen	JL	VS20-05009 B1	1+	1.0	1.0	2.0	2.4	2.0	2.4	5	5	None	Signals are much easier to visualize than old stain, background is cleaner.
Pizzi	KB	OSW20-2875 A1	1+	1.0	1.1	2.1	2.0	2.2	2.3	5	5	Old stain.	New stain - signals much more clear, easier to enumerate.
Libby	KB	IS20-00946 A1	–	–	1.0	–	2.0	–	2.0	–	–	Stain failed. Sent for FISH	New stain - old stain failed.
GI Cases													
Libby	JL	CRS20-1984 C1	2+	1.0	1.0	2.0	2.2	2.0	2.2	5	5	None	Signals are larger and more distinct than on old stain.
Pizzi	NP	NS20-03964 A1	–	–	–	–	–	–	–	–	–	Both Her2 DISH and zHer2 DISH failed due to high background. The specimen was liver lesion, core bx. This case was not sent for FISH. Due to fail, the slides were not scored.	Both Her2 DISH and zHer2 DISH failed due to high background. The specimen was liver lesion, core bx. This case was not sent for FISH. Due to fail, the slides were not scored.