Microfluidic Immunohistochemistry for Rapid Detection of Diagnostic and Predictive Biomarkers on Non–Small Cell Lung Carcinoma

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Background

Lung cancer is the most lethal tumor world-wide. Immunohistochemistry (IHC) is an essential tool in oncologic pathology. Considering the increasing cancer incidence, a concomitantly accurate and time-efficient IHC is clinically highly relevant. The automated microfluidic staining device enables for a precisely controlled immune-reaction to take place in an extremely short incubation time; typically, a complete microfluidic IHC lasts less than 12 minutes on frozen tissue section.

Objective

We aimed to develop and validate a microfluidics IHC for fast and accurate biomarkers detection on formalin fixed paraffin-embedded (FFPE) non-small cell lung carcinomas (NSCLC) - the most common lung cancer type.
Here, we focus on the first part of the study aiming at optimizing the microfluidic protocols.

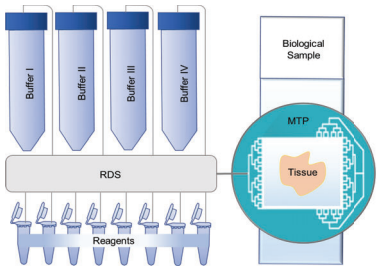
Methods

Each protocol step - from epitope retrieval to counterstaining - was automatized and optimized on the microfluidic staining device **(Figure 1)** for all biomarkers on representative FFPE NSCLC specimens to reach high-quality staining equivalent to a routinely used Ventana BenchMark ULTRA automated stainer.

Results

The optimized microfluidic IHC protocol achieved an analytical performance comparable to standard staining on NSCLC for (i) differential diagnosis **(Figure 2)** into adenocarcinoma (TTF1/CK7) or squamous cell carcinoma (p40/CK5-6) and (ii) prediction of immuno-therapy response (PD-L1) **(Figure 3)**. Concomitantly, the total process time was shortened down to twenty minutes - including the epitope retrieval pre-staining step, with a time reduction up to nine folds **(Table 1)**.

FIGURE 1. Microfluidic device schematic representation



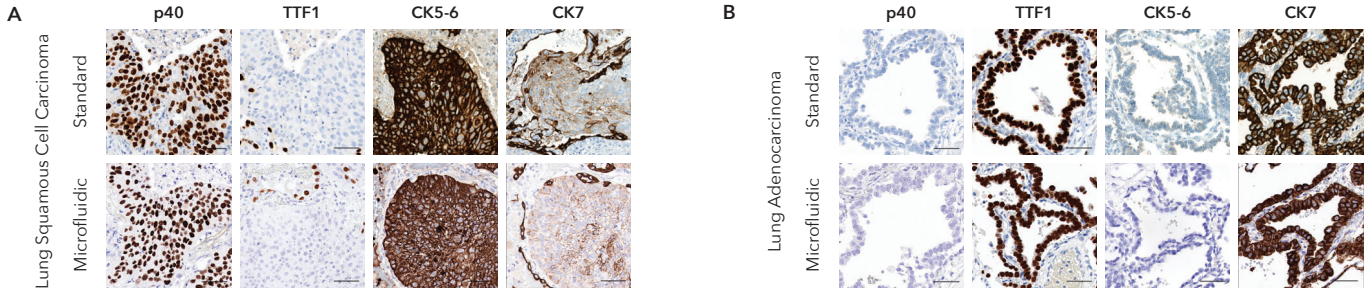
The automated microfluidic staining device is a pressure-driven system allowing for fast and uniform delivery of reagents via the reagent delivery system (RDS) to a reaction chamber created at the interface between a microfluidic chip (MTP) and the microscope slide carrying the tissue sample.

TABLE 1. Optimized microfluidic IHC protocols in comparison with standard assays on FFPE NSCLC

Marker		p40		TTF1		CK5-6		CK7		PD-L1	
Abl Clone		BC28		SP141		D5/16B4		SP52		E1L3N	IHC411
Abl Dilution		1:100	1:50	1:50	RTU	RTU		RTU		1:100	1:50
Staining Device		**STD**	**MTP**	**STD**	**MTP**	**STD**	**MTP**	**STD**	**MTP**	**STD**	**MTP**
Protocol Steps	HIER	24	5	56	5	36	5	4*	10	56	10
	H_2O_2	4	--	4	--	4	--	4	--	4	--
	Abl	32	4	12	4	32	1	16	1	32	4
	Linker	8	--	8	--	8	--	8	--	8	--
	AblI	8	4	8	4	8	1	8	1	8	4
	DAB	8	2	8	2	8	2	8	2	8	2
	$CuSO_4$	4	**	4	--	4	--	4	--	4	**
	HTX	8	0.25	8	0.25	8	0.25	8	0.25	8	0.25
Total Time (min)		**173**	**26**	**184**	**26**	**188**	**20**	**124**	**25**	**220**	**31**

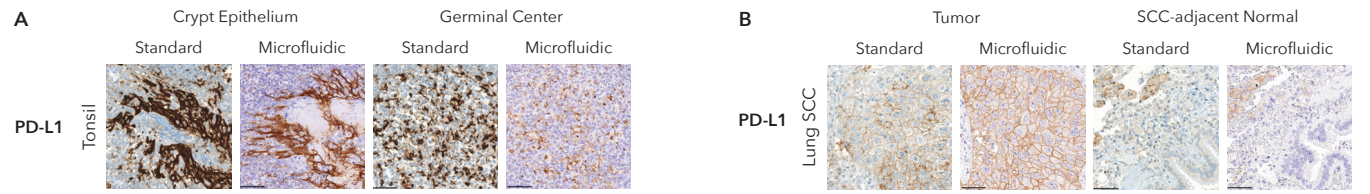
Optimized microfluidic IHC protocols (MTP) for p40, TTF1 , CK5-6, and CK7 on FFPE NSCLC. Incubation time for all protocol step and primary antibody dilutions are shown as compared to routinely used standard (STD) protocols. RTU: ready to use; HIER: heat-induced epitope retrieval; H_2O_2: oxygen peroxidase; AbI/II: primary/secondary antibody; DAB: 3,3'-diaminobenzidine; $CuSO_4$: copper sulfate; HTX: hematoxylin. *PIER: proteolytic-induced epitope retrieval **$CuSO_4$ post-staining manual incubation for 30sec.

FIGURE 2. Analytical performance comparison for p40, TTF1, CK5-6 and CK7 IHC staining by standard versus microfluidic assay on FFPE NSCLC tissues



Representative IHC staining for p40, TTF1, CK5-6 and CK7 by standard (top panels) or microfluidic-based (bottom panels) assay on FFPE lung squamous cell carcinoma (A) and adenocarcinoma (B). Scale bar 50 µm.

FIGURE 3. Analytical performance comparison for PD-L1 IHC staining by standard versus microfluidic assay on FFPE tonsil and NSCLC tissues



(A) Representative IHC staining for PD-L1 by standard or microfluidic-based assay on FFPE tonsil crypt epithelium (left panels) and germinal center (right panels). (B) Representative IHC staining for PD-L1 by standard or microfluidic-based assay on FFPE lung squamous cell carcinoma (left panels) and normal adjacent tissue (right panels). Scale bar 50 µm.

Conclusions and Future Directions

The microfluidic IHC resulted in fast automated high-quality staining for all assessed markers on NSCLC. Subsequent clinical validation on a large cohort will provide a diagnostic tool for biomarkers detection in a turnaround time far beyond the existing automated stainers.

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