A Comparison between Manual and Automated Q-STAIN® X Immunostaining in Skin Cancer

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PURPOSE

Immunohistochemistry (IHC) demands for intraoperative surgery to support definitive surgical care have increased during a time of staff shortages in histology laboratories[1,2]. These added demands are driving the need to shift from manual IHC testing to automated IHC to increase the volume of slides tested, enhance the standardization of staining protocols while alleviating labor pressures and decreasing processing time[2,3]. Rapid automated IHC testing may help overcome insufficient human resources and help reduce human error while decreasing test turnaround times and improving overall laboratory efficiency. This combination of rapid automated IHC testing and quality results on frozen tissue sections may thereby benefit intraoperative surgeries such as Mohs micrographic surgery (MMS)[4]. Melanocytic markers such as Melanoma antigen recognized by T cells (MART-1) and SRY-related HMG-box 10 (SOX10) are often used to identify melanoma during extraction of malignant tissue during MMS[5,6]. MART-1 and SOX10 antibodies stain malignant melanocytes and normal background melanocytes that serve as internal controls. MART-1 is a cytoplasmic marker commonly used as an adjunct to Hematoxylin and Eosin (H&E) frozen sections to detect junctional and focal dermal melanocytic proliferation on frozen sections[6]. SOX10 is a nuclear marker and has historically been considered the standard for desmoplastic and spindle cell melanomas[7,8]. Cytokeratin 5 (CK5) is a high molecular weight keratin expressed in squamous cell epithelium, myoepithelial cells of the breast, mesothelium, and the basal cells of the prostate. CK5 is a rabbit monoclonal antibody (mAb) that binds specifically to basal and squamous cell carcinoma of skin, and it also binds to squamous cells of epidermis and hair out-root sheath cells, sebaceous glands, and basal cells of eccrine and apocrine ducts[9]. These properties make CK5 antibody a reliable marker for both basal cell (BCC) and squamous cell carcinoma (SCC) of skin[9]. In the present study, we aimed to demonstrate concordant staining results when comparing manual staining to Q-STAIN®X (QSX) autostainer results when using Novodiax (Hayward, CA) anti-MART-1, anti-SOX10, and anti-CK5 antibodies with polymerized Horseradish Peroxidase (poly-HRP) technology on frozen skin cancers (melanoma and non-melanoma). The staining results were reviewed and graded by an in-house pathologist to assess staining quality.

DESIGN

Poly-HRP combined with MART-1, SOX10, and CK5 antibodies were used for manual and automated IHC staining. Frozen tissues were cut 4 microns in thickness and fixed with reagent grade acetone with an incubation time of 1 minute excluding melanoma tested with SOX10. 10% Neutral buffered formalin (NBF) was used as the fixative for SOX10 staining. Slides were then transferred to a phosphate buffered saline (PBS) wash buffer. For the IHC protocol: Blocker was applied to tissue samples for 1 minute, primary antibody (Mart-1, SOX10, CK5) incubation time was 4 minutes, Enhancer incubation time was 3 minutes, and 3,3'-Diaminobenzidine (DAB) chromogen incubation time was 2:30 minutes. Finally, tissues were counterstained with Mayer's hematoxylin with an incubation of 1 minute, dehydrated in alcohol, cleared in Xylene, and permanently mounted (Figure 1). Slide staining results were reviewed by a board-certified pathologist with scores ranging from 0-4.5 with scores of 4.0 or higher considered optimal. Non-specific background staining was also graded with scores ranging from 0-1 with 0 indicating no background and scores below 0.5 considered optimal.

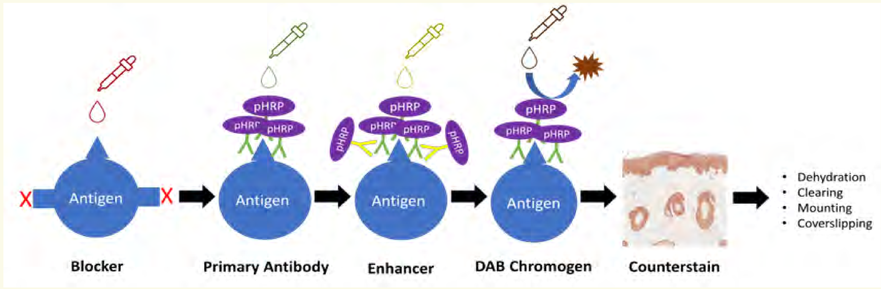

Figure 1. IHC protocol schematic.

Steps	Manual IHC Time (minutes)	Automated IHC Time (minutes)
Fixation	1	1
Wash Buffer	0.25	0.25
Blocker	1	1
Wash Buffer	0.25	0.25
Primary Antibody	4	4
Wash Buffer	0.25	0.25
Enhancer	3	3
Wash Buffer	0.25	0.25
Chromogen	2.5	2.5
Water	0.25	0.25
Counterstain	1	1
Dehydration, Clearing, Coverslip	<1	<1
TOTAL IHC RUN TIME	<15 minutes	<15 minutes

Table 1. IHC run times for Manual and Automated methods. Red outline indicates IHC Steps performed onboard the QSX autostainer.

Figure 2. Q-STAIN X may be used independently or as a companion to other automated staining devices to support high volume testing facilities. Its capacity to process 10 frozen, formalin-fixed, or H&E slides with rapid test turnaround time and its ability to achieve standardized results in clinical settings.

Tissue	Testing Method	Antibody	Staining Quality Grade/Result	Background Grade/Result
Melanoma	Manual	MART-1	4.5/Optimal	0.1/Optimal
Melanoma	QSX	MART-1	4.5/Optimal	0.1/Optimal
Melanoma	Manual	SOX10	4.5/Optimal	0.1/Optimal
Melanoma	QSX	SOX10	4.5/Optimal	0.1/Optimal
Non-Melanoma	Manual	CK5	4.5/Optimal	0.1/Optimal
Non-Melanoma	QSX	CK5	4.5/Optimal	0.1/Optimal

Table 2. Tissue type, IHC testing method, antibody, and pathologist recorded grades of staining quality and nonspecific background.

FINDINGS

Manual staining was completed in less than 15 minutes demonstrating increased testing efficiency compared to conventional IHC techniques with turnaround times ranging from 30-90 minutes. QSX automated staining was completed in less than 20 minutes (Table 1). All tissues tested demonstrated equivalent staining quality based on pathologist review and grading across the manual and automated immunostaining methods (Table 2) (Figure 3).

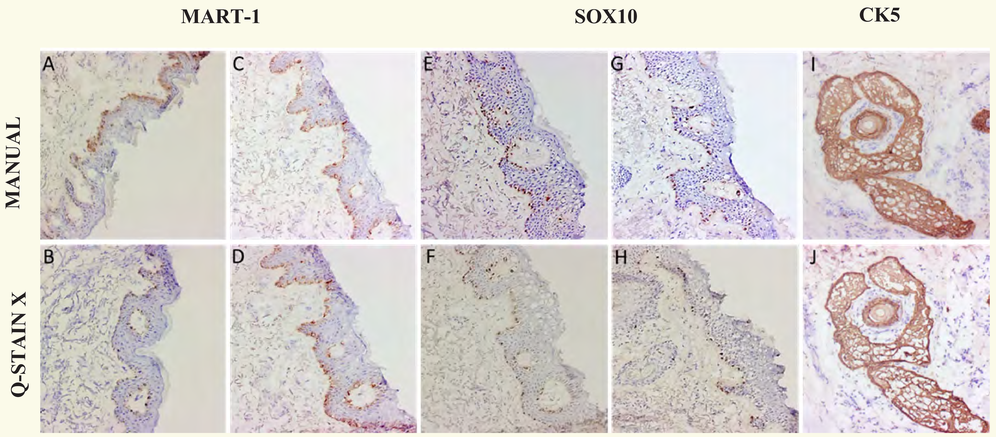

Figure 3: A,C- Manual staining of Melanoma tissue with MART-1, 10X. **B,D**- Q-STAIN X staining of Melanoma tissue with MART-1, 10X. **E,G**- Manual staining of Melanoma tissue with SOX10, 10X. **F,H**- Q-STAIN X staining of Melanoma tissue with SOX10, 10X. **I**- Manual staining of SCC tissue with CK5, 10X. **J**- Q-STAIN X staining of SCC tissue with CK5, 10X.

SUMMARY

The rapid QSX autostainer turnaround time, consistent staining quality, and minimal need for operator interaction support its use for clinical intraoperative applications such as MMS. Results indicate the clinical utility of standardized and consistent automated IHC in intraoperative testing as seen with MART-1, SOX10, and CK5 to aid in rapid identification and removal of malignant tissues.

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