

# Mapping Basal Cell and Squamous Carcinoma By 10 Min CK5 Direct Immunohistochemistry on Frozen Section Skin Tissues during Mohs Micrographic Surgery

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## Summary

In this study, a novel one-step CK5 direct immunohistochemistry (IHC) was used to map basal cell and squamous cell carcinoma on frozen section skin tissues during Mohs micrographic surgery (MMS). Total 64 frozen section skin specimens from 21 patients who have either basal cell carcinoma or squamous carcinoma were used for this study. The results showed that this one-step CK5 direct IHC can detect both basal cell(BCC) and squamous carcinoma(SCC) on skin frozen section tissues with minimal background stain. This one-step CK5 direct IHC method is simple to use, and it is ultra-fast comparing with current IHC methods. The entire procedures of this one-step CK5 direct IHC can be finished within 10 min with no need of expensive machine and it can be performed either at room temperature or on a slide warmer at 30° C. This novel one-step CK5 direct IHC method is potentially a valuable tool for accurately mapping tumor cells on frozen section skin tissue during MMS and to improve the outcome of surgical treatment of skin cancer

## Introduction

Mohs micrographic surgery (MMS) is a standard treatment method for skin cancers in aesthetic and functional sensitive head-neck regions, especially for nonmelanoma skin cancers, such as two most common skin cancers, basal cell carcinoma and squamous cell carcinoma (1, 2). It offers both tissue sparing and the lowest possible recurrence rates for excised nonmelanoma skin cancers. The results of MMS treatment are highly dependent on accuracy of tumor mapping and identification of tumor cells at the tissue margins. For most basal cell carcinoma, it is relatively reliable to identify large tumor cell nests on hematoxylin and eosin (HE) stained frozen section tissue slides for well-trained Mohs surgeon, dermatologists and dermatopathologists (1,2). However, in practice, it may be difficult or impossible to identify single or small cell nests of tumor, and to differentiate inflammatory cells versus tumor cells, and stromal reactions versus tumor. When in doubt, the main option is to excise more tissue, leading to additional cost and potentially unpleasant or disfiguring surgery for the patient.

Immunohistochemistry (IHC) is a proven method for clarifying the diagnosis in ambiguous sections, but the use of current IHC method is not practical in Mohs surgery because of the time required to complete the staining process (3 to 8). In addition, traditional IHC involves multiple amplification steps, and it is time consuming with the potential for error or background artifact with each step of the process (3 to 8). In this report, we describe our experience with a novel simplified one-step IHC using horseradish peroxidase (HPR) polymers conjugated primary antibodies. This process is extremely fast and sensitive. The whole procedure can be completed within 10 minutes without need for expensive and specialized equipment.

## Materials and Methods

Total 21 patients who have either basal cell or squamous cell carcinoma were selected for the study (Table 1). A rabbit monoclonal antibody to CK5 conjugated with HRP polymers (Novodiex Inc., Hayward California), was used for this study. CK5 is high molecular weight keratin, a reliable marker for both basal cell and squamous cell carcinoma of skin. Anti-CK5 rabbit monoclonal antibody (mAb) binds specifically to basal and squamous cell carcinoma of skin and it is also binds to squamous cells of epidermis and hair out-root sheath cells, sebaceous glands and basal cells of eccrine and apocrine ducts (9 to11). This one step IHC protocol was performed according the manufacturer's instructions (Table 2). The frozen skin tissues from Mohs surgery margins were cut at 5 micron thickness in three levels for HE stains and an additional cut was used for one-step CK5 direct IHC stains at same time. Total 64 specimens from patient's Mohs tissue margins were stained with both hematoxylin and eosin (HE) and one-step CK5 direct IHC during surgeries. The one-step CK5 direct IHC stains were performed at 30° C on manufacture's slide warmer. The IHC results were read with HE stained frozen section slides at same time. The left-over tissues after frozen sections were fixed with 10% formalin for paraffin embedded (FFPE) permanent section for HE stains. The results of intraoperative IHC and frozen section HE stains were also compared with the results based on FFPE permanent section HE stained slides.

## Results

The one-step CK5 direct IHC procedures are performed as summarized in table 2. The procedures are simple and easy to operate and the all procedures were finished within 10 min following the manufacture's protocol, which allowed all patient's Mohs surgeries finished within usual time without significant delaying after addition of the intraoperative one-step CK5 direct IHC stains. All the one-step CK5 direct IHC were performed on the manufacture's slide warmer at 30° C.

The results of intraoperative diagnosis by HE stains and CK5 direct IHC are summarized in table 3. The results of intraoperative diagnosis for BCC based on by HE stains and CK5 direct IHC stains are all confirmed with permanent section diagnosis (Table 4 and Figure 1, 3A, 3B, 3C and 3D) except two specimens. These two specimens showed BCC on HE stained permanent sections of FFPE tissues that were not seen for BCC on intraoperative frozen section HE stains and CK5 direct IHC stains. The reason for this discrepancy are due to deeper recuts of the left-over tissues after FFPE. The original intraoperative frozen section HE and CK5 direct IHC stained slides were reviewed and the original diagnoses were correct. The results for of intraoperative diagnosis based on HE stains and CK5 direct IHC stain were all confirm by HE stains of permanent section of FFPE tissues (Figure 1 and table 3 and 4).

## Results

The CK5 direct IHC stains of normal frozen section skin tissues are shown in Figure 2, and normal epidermal squamous cells, epithelia sebaceous glands, out-root sheath of hair follicles and basal layer keratin of eccrine glands are highlighted with brown color by CK5 direct IHC.

The tumor cells of basal cell carcinoma frozen section slides are highlighted in brown colors after CK5 direct IHC stains, that can be easily distinguished from normal skin structures, inflammatory cells and reactive stroma on frozen section slides (Figures 3A, 3B, 3C, and 3D). The CK5 direct IHC stain also can help to identify the crushed tumor cell nests during frozen section cutting process (Figure 3D).

The tumor cells of squamous cell carcinoma frozen section slides are highlighted in brown colors after CK5 direct IHC stains, that can be easily distinguished from normal skin structures on frozen section slides (Figures 4A and 4B).

The background stain of CK5 direct IHC is minimal and all stromal cells and inflammatory cells caused by previous surgical procedures were not stained with CK5 direct IHC.

**Table 1.** Characteristics of 21 Patients for Intraoperative CK5 Direct IHC Study

<b>Patients</b>	<b>Age</b>	<b>Sex</b>	<b>Diagnosis</b>	<b>Location</b>
1	49	M	BCC	Left submandubular
2	70	M	BCC	Right lateral forehead
3	73	F	BCC	Left eyebrow
4	69	F	BCC	Upper forehead
5	76	F	BCC	Left nasal crease
6	67	F	BCC	Nasal bridge
7	73	M	BCC	Left parietal scalp
8	72	M	BCC	Right forehead
9	78	M	BCC	Right upper lip
10	76	M	BCC	Right posterior pinna
11	68	M	BCC	Left lateral forehead
12	80	M	SCC	Left triangular fossa
13	77	M	SCC	Right ear helix
14	74	M	BCC	Right lower lip
15	98	F	SCC	Right temple
16	79	F	BCC	Left upper lip
			SCC	Left zygomatic cheek
17	77	M	SCC	Right temple
18	87	F	BCC	Right nasal bridge
19	88	M	BCC	Right upper forehead
20	66	F	BCC	Right nasal bridge
21	67	F	BCC	Right upper forehead

**Table 2: 10 min Direct IHC Protocol for Frozen Section Tissue**

<b>• Procedure</b>	<b>Time in minutes</b>
1. Wash twice with PBS-T	0.2
2. Blocking with common IHC blocker	1
3. Add polyHRP –anti-CK5 conjugate	3
4. Wash twice with PBS-T	0.2
5. Add DAB working solution	3
6. Wash twice with PBS-T or tap water	0.2
7. Hematoxylin counterstaining	0.30
8. Wash twice with PBS-T or tap water	0.20
9. Mounting with aqueous medium + coverslip	0.50
<b>• Total</b>	<b>10</b>

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**Table 3.** Summary of total cases of basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) and specimen that frozen section H/E diagnosis and CK5 direct IHC were performed during Mohs surgeries

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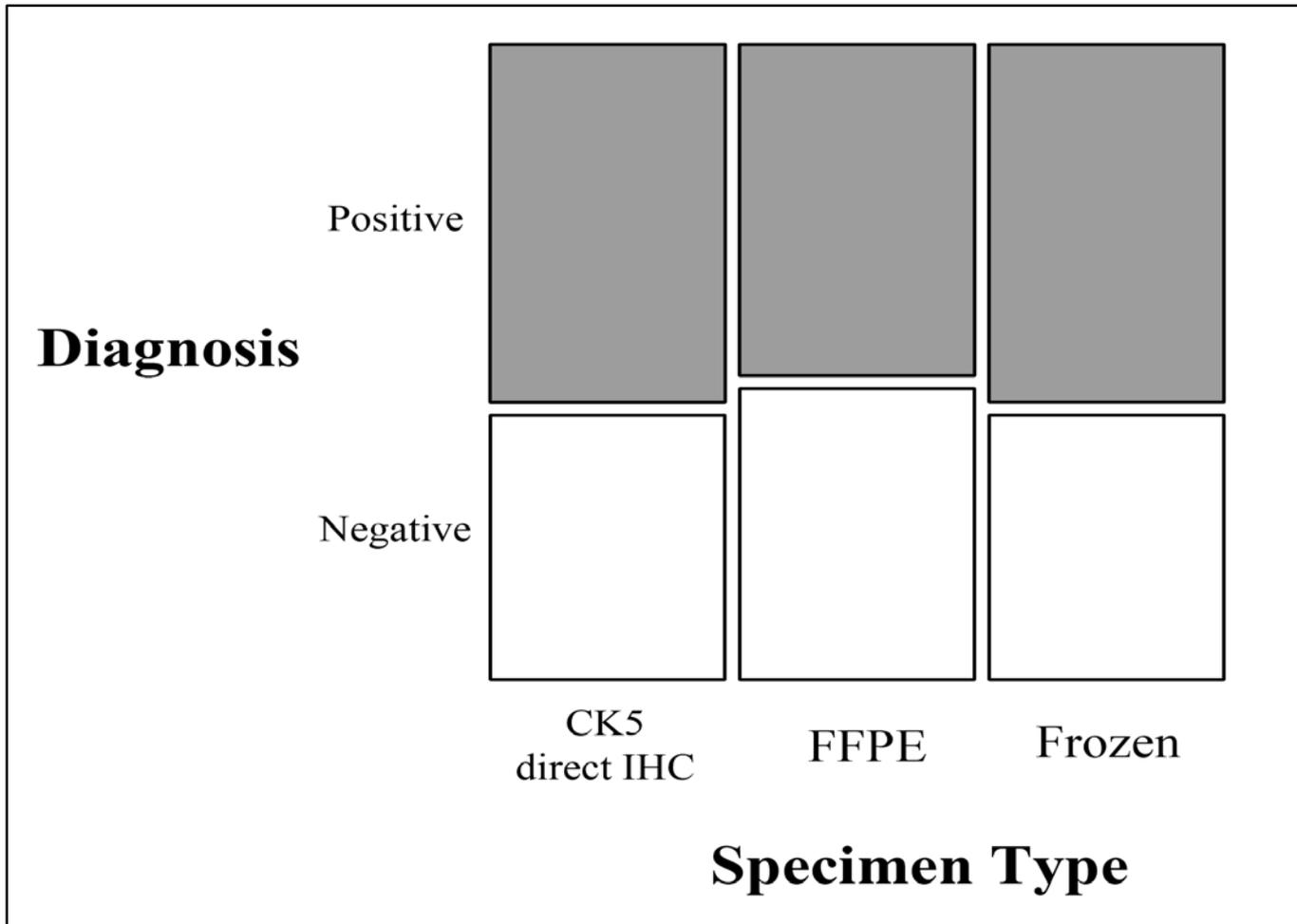
<b>Biopsy Diagnosis</b>	<b>Total Cases for Mohs Surgeries</b>	<b>Frozen Section Diagnosis by H/E Stains</b>	<b>CK5 direct IHC Stains</b>	<b>Total</b>
BCC	16	47	47	110
SCC	6	17	17	40
Total	22	64	64	150

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**Table 4.** Summary of results by frozen section diagnosis and CK5 direct IHC results for basal cell carcinoma (BCC).

<b>Diagnosis</b>	<b>Frozen Section H/E</b>		<b>CK5 direct IHC Stain</b>		<b>FFPE Tissue</b>	
	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>
Positive for BCC	20	42.6	20	42.6	22	46.8
Negative for BCC	27	57.4	27	57.4	25	53.2

Pearson's Chi-squared test of independence at an alpha-value of 0.05 was performed. There was no significant difference between the ( $\chi^2=0.2303$ ,  $df = 2$ ,  $p = 0.8912$ ).



**Figure 1.** Mosaic plot of basal cell carcinoma diagnosis results by specimen type

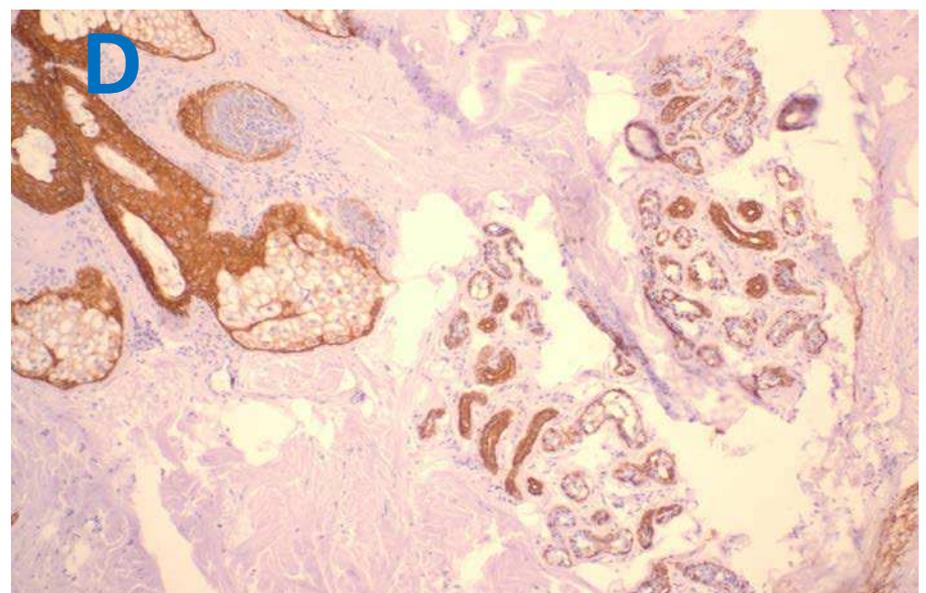
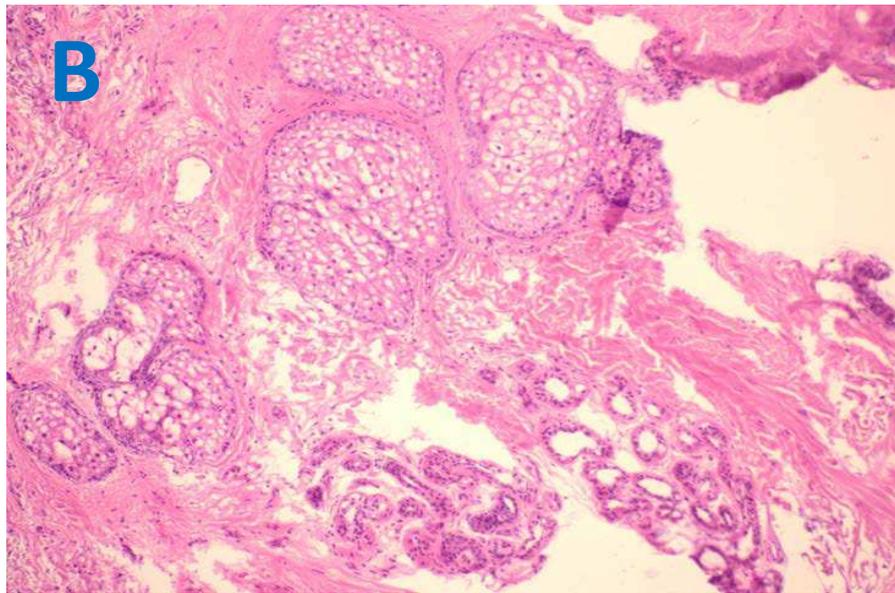
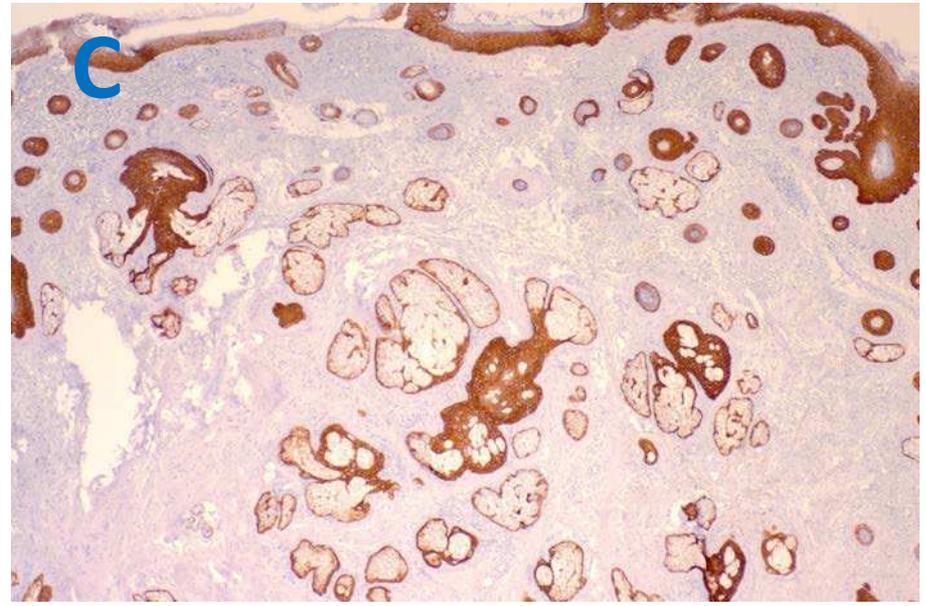
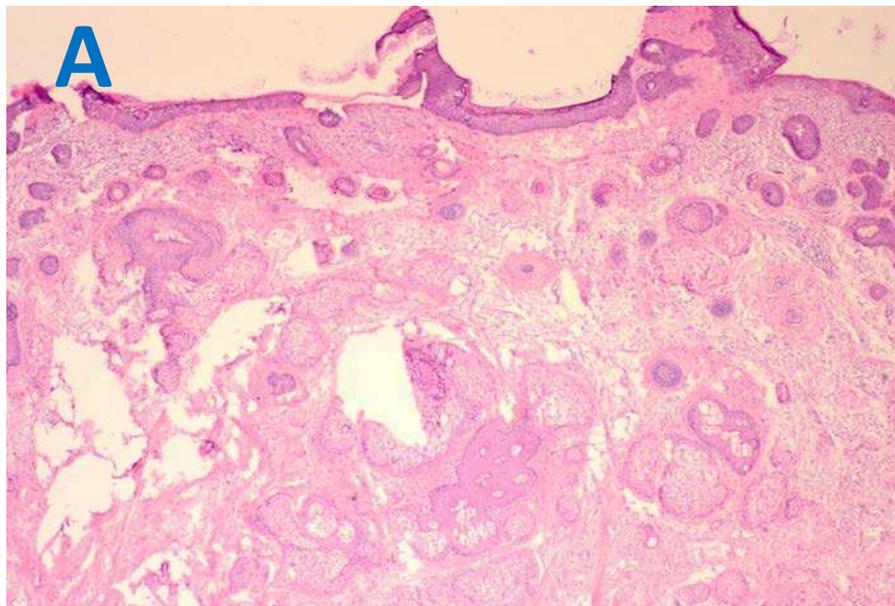
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**Table 5.** Summary of results by frozen section diagnosis and CK5 direct IHC results for squamous cell carcinoma (SCC).

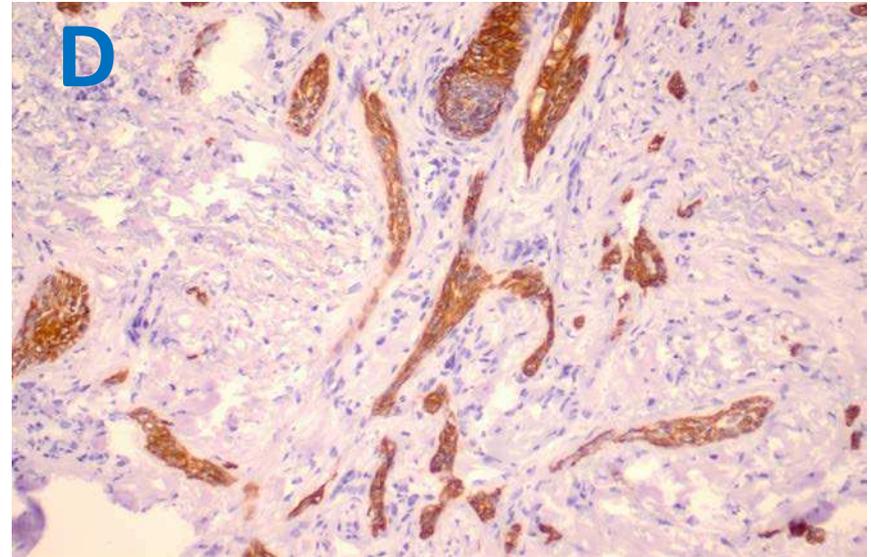
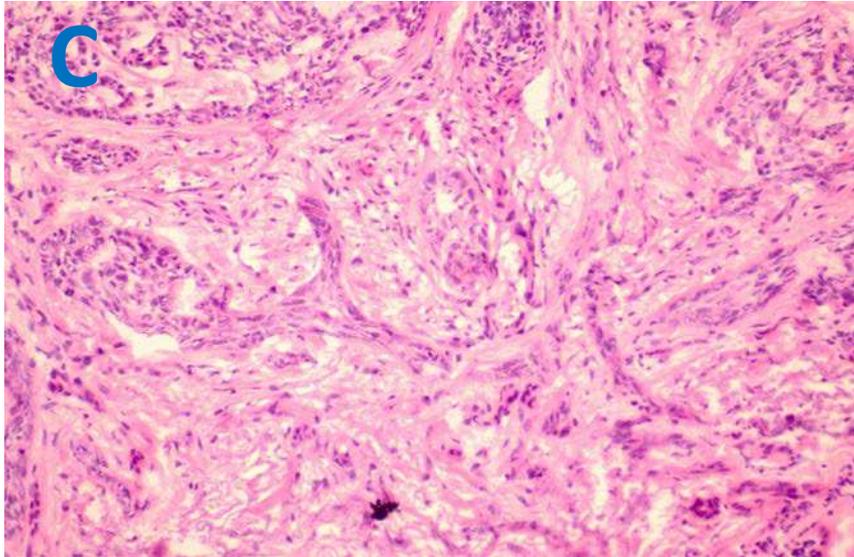
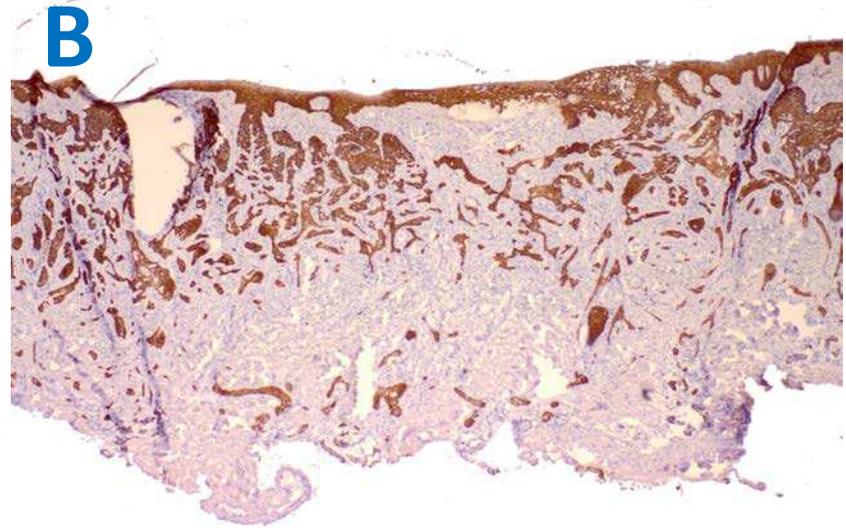
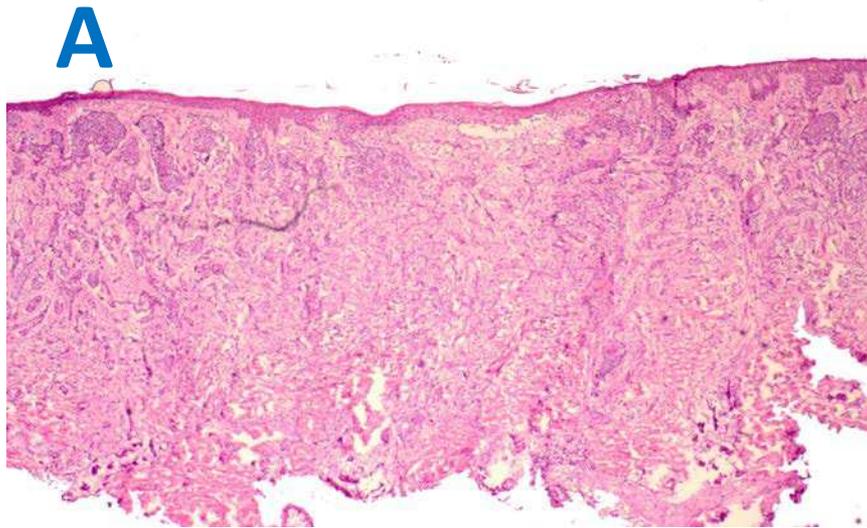
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<b>Diagnosis</b>	<b>Frozen Section H/E</b>		<b>CK5 direct IHC Stain</b>		<b>FFPE Tissue</b>	
	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>	<b>N</b>	<b>%</b>
Positive for SCC	6	35.3	6	35.3	6	35.3
Negative for SCC	11	64.7	11	64.7	11	64.7

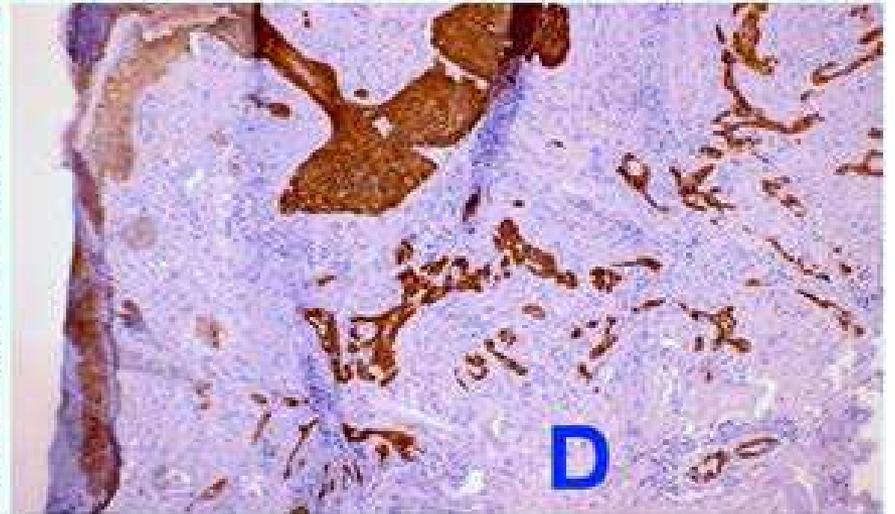
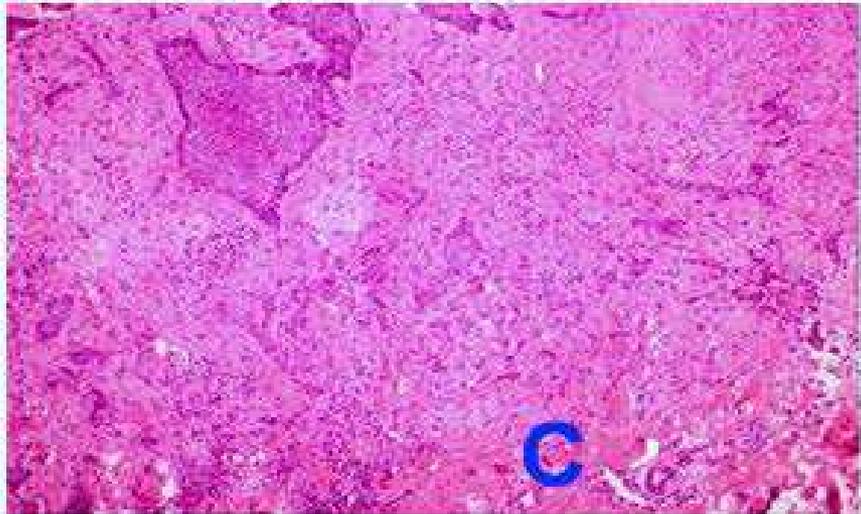
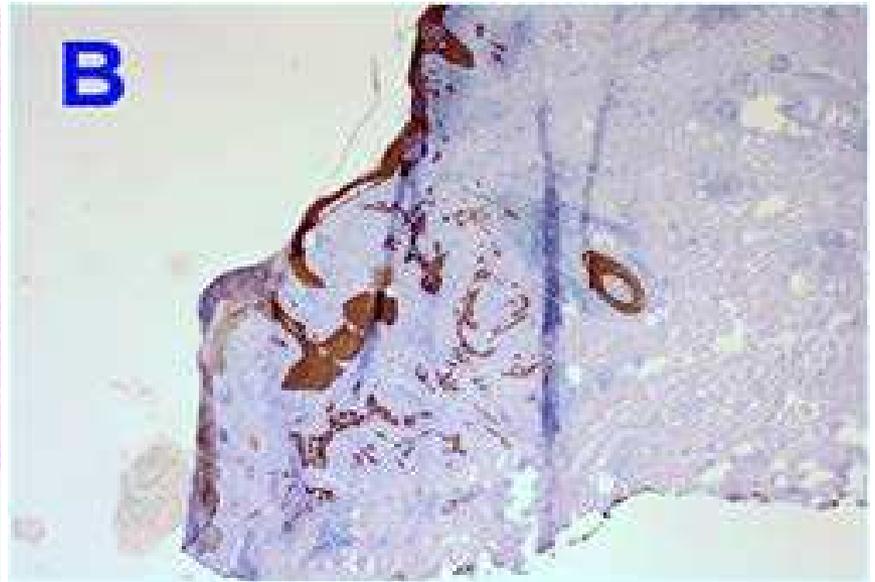
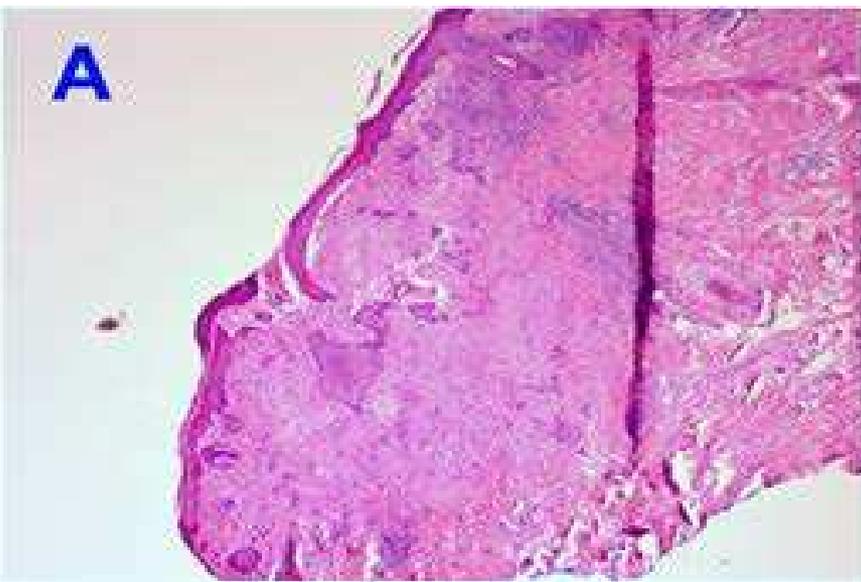
Pearson's Chi-squared test of independence at an alpha-value of 0.05 was done. There was no difference between the ( $\chi^2=0$ , df = 2, p = 1.0).



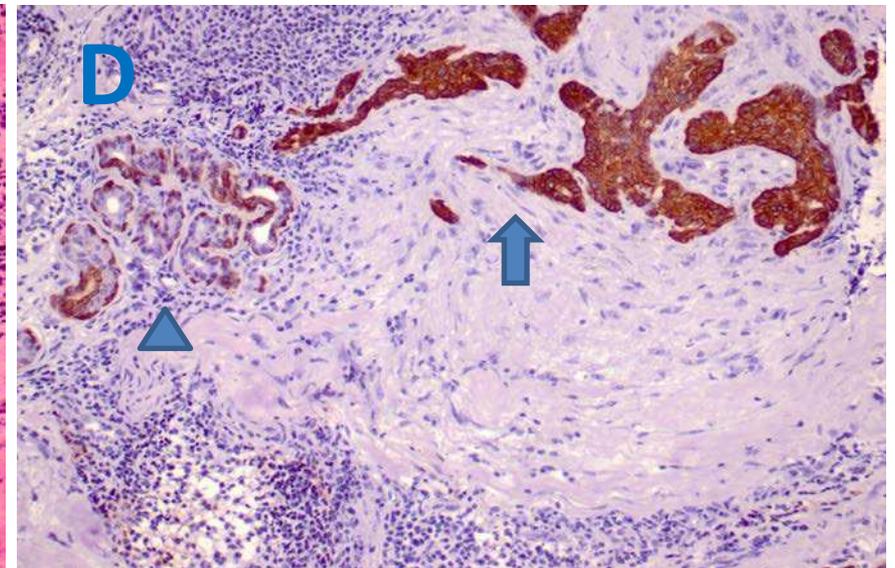
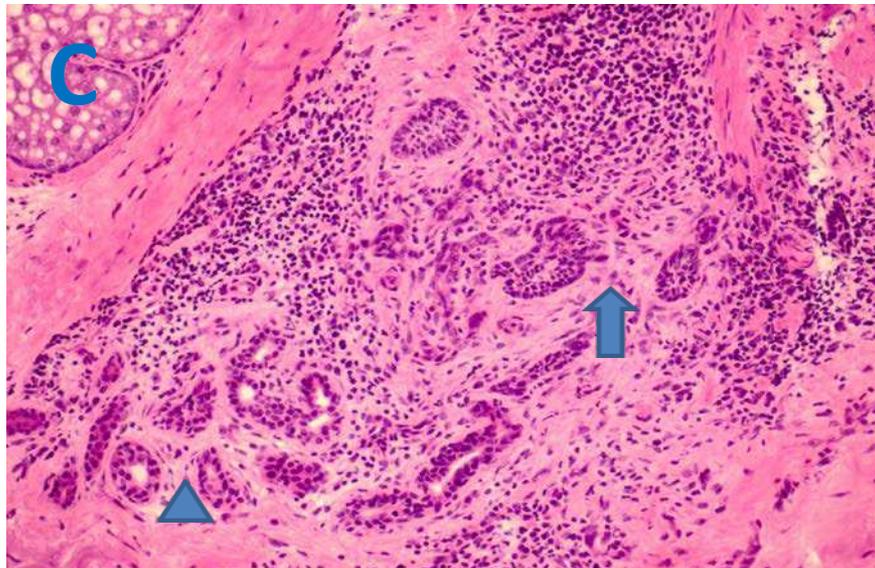
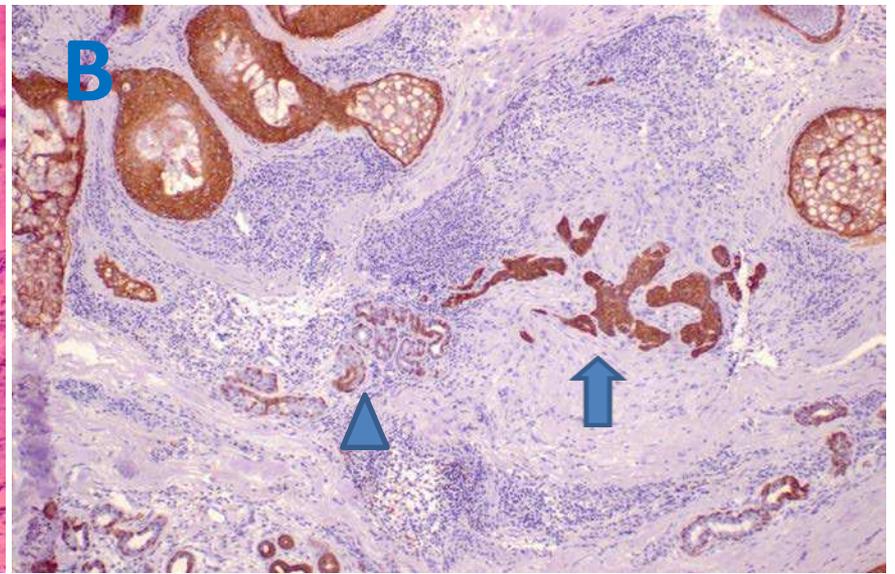
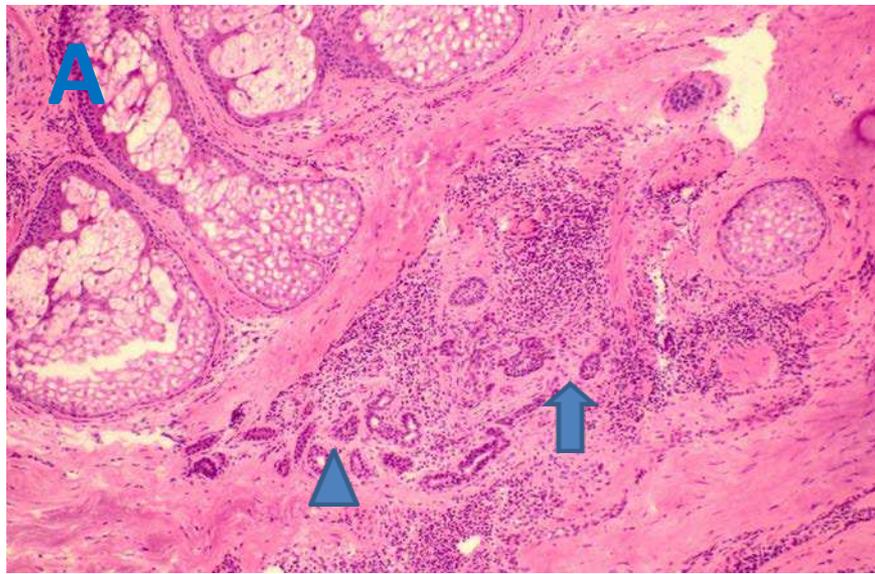
**Figure 2.** CK5 direct IHC stain of normal frozen section skin tissue. A and B, HE stain; C and D, CK5 direct IHC stain. Normal skin epidermal squamous cells, sebaceous glands, out-root sheath of hair follicles, and basal layers of eccrine glands are highlighted with CK5 direct IHC.



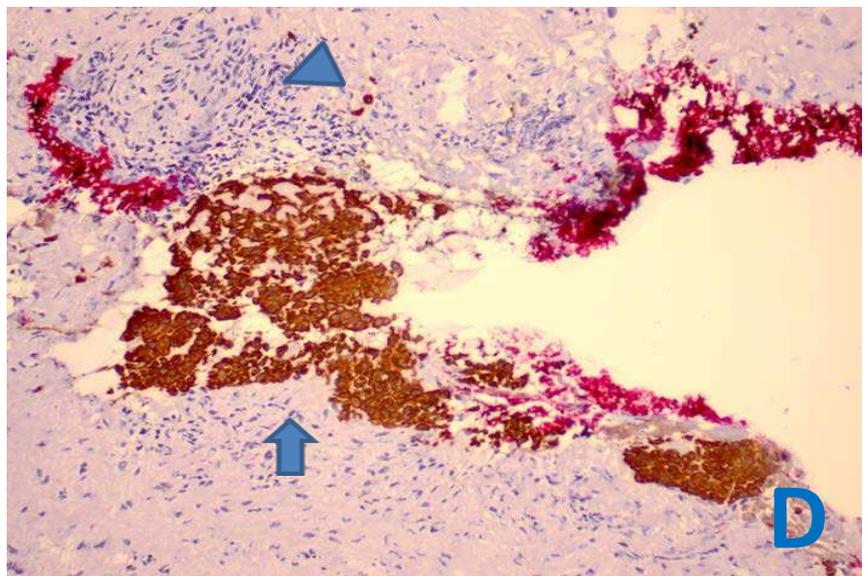
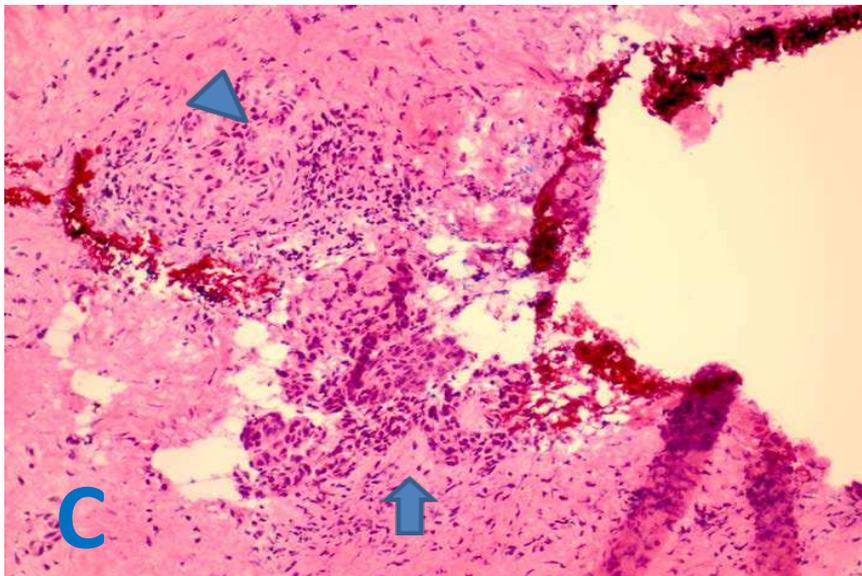
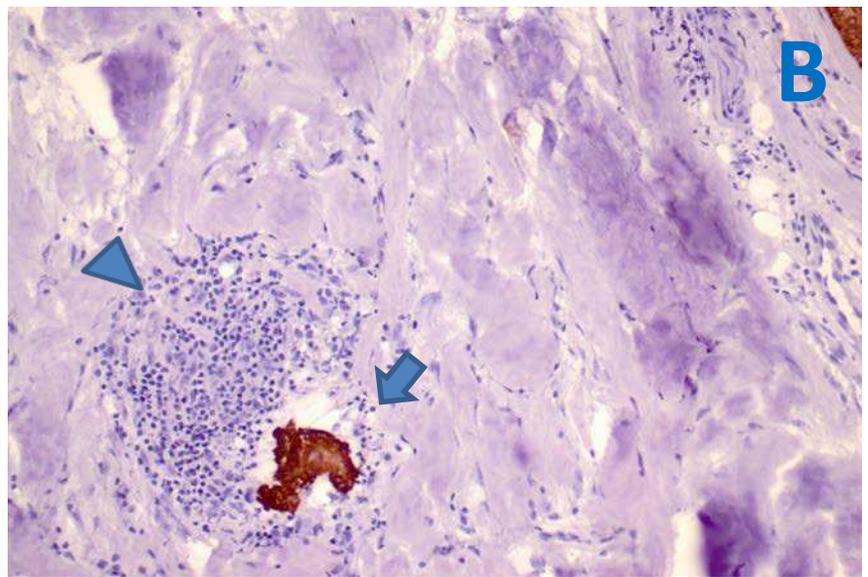
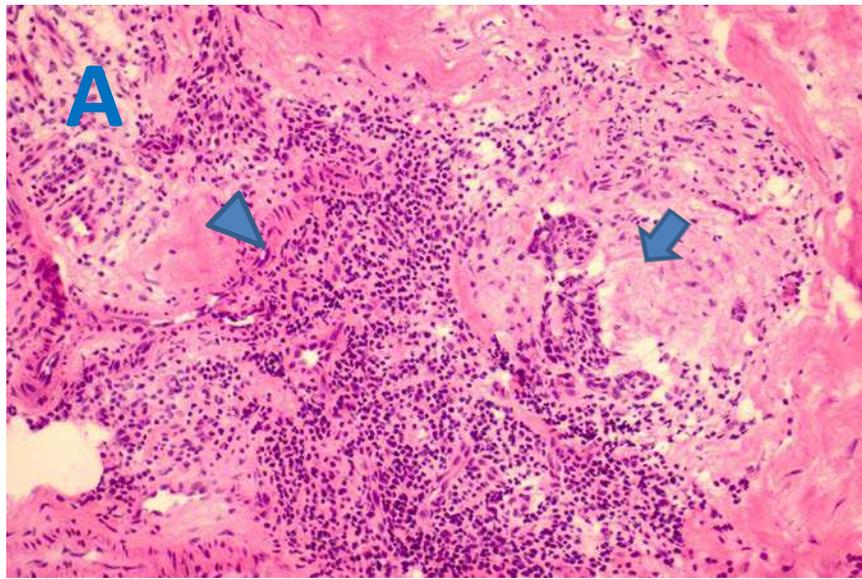
**Figure 3A.** CK5 direct IHC stain of infiltrating basal cell carcinoma. A and C, HE stain; B and D, CK5 direct IHC stain. The infiltrating tumor cell nests of basal cell carcinoma are highlighted with CK5 direct IHC stain. A and B, low magnification (2X); C and D, high magnification (20X)



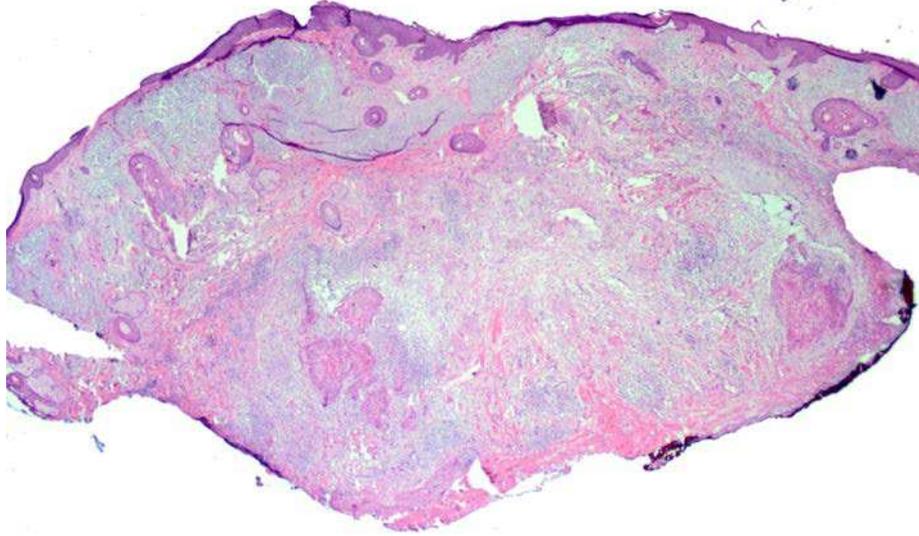
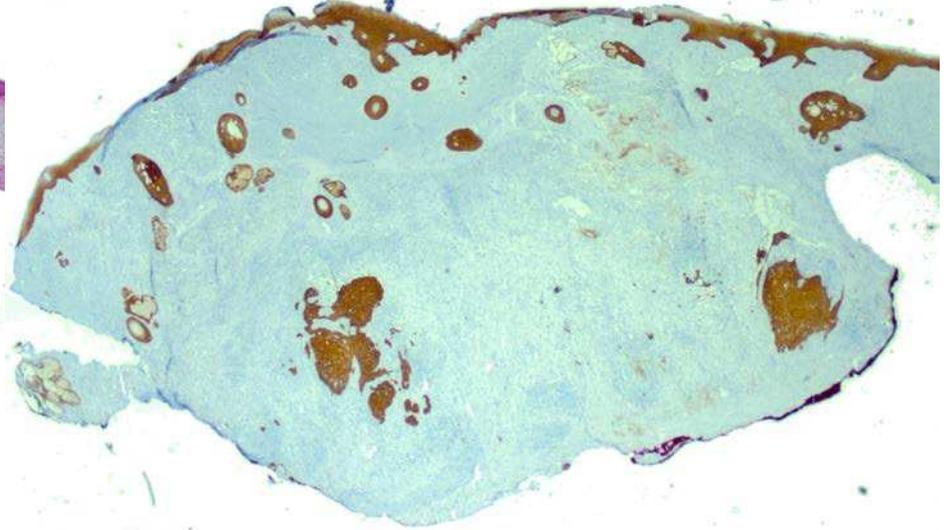
**Figure 3B.** CK5 direct IHC stain of nodular and infiltrating basal cell carcinoma. A and C, HE stains; B and D, CK5 direct IHC stains. The infiltrating tumor cell nests of basal cell carcinoma are highlighted with CK5 direct IHC stain. A and B, low magnification (2X); C and D, high magnification (20X).



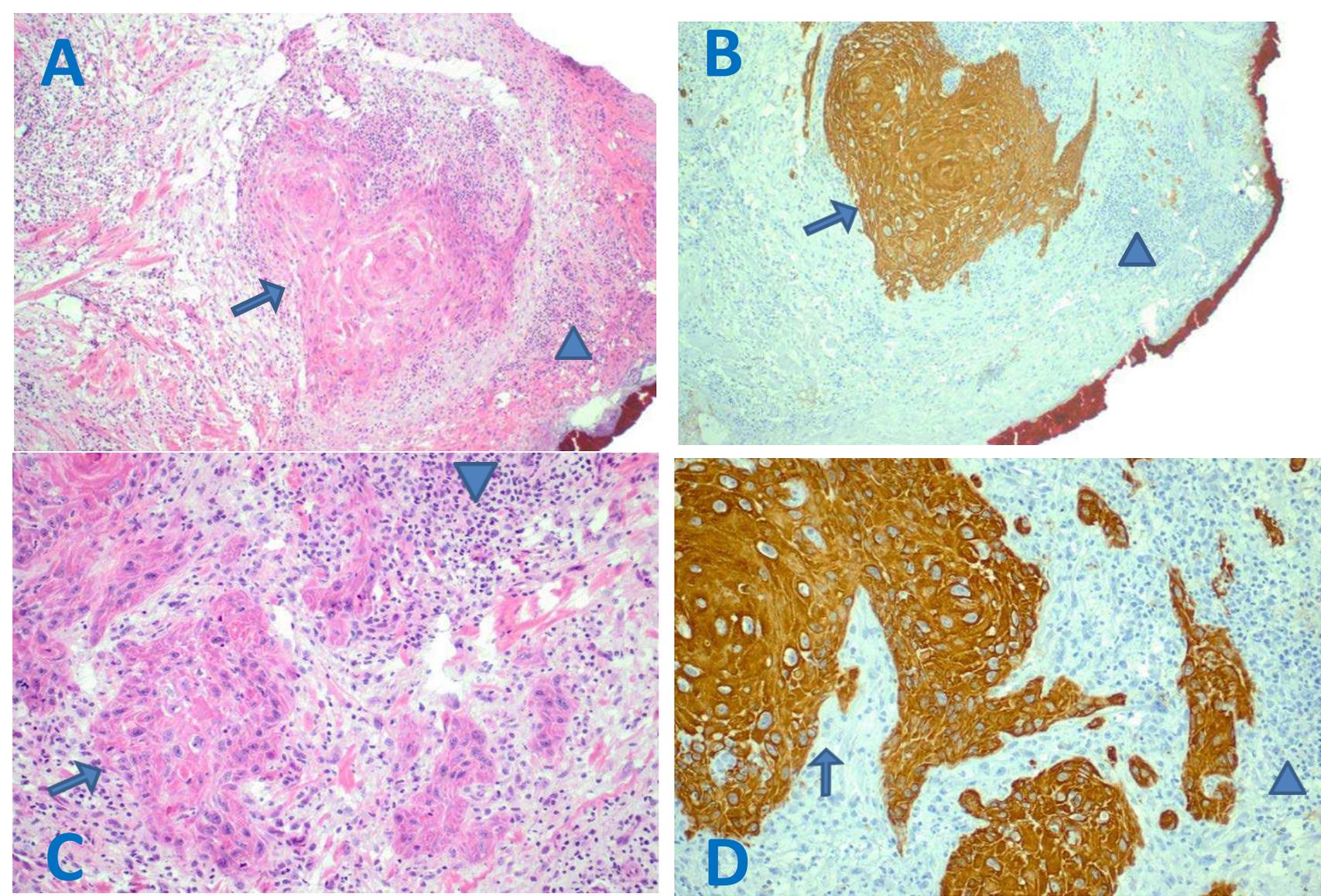
**Figure 3C.** Direct CK5 IHC stain of nodular and infiltrating basal cell carcinoma. A and C, HE stains; B and D, CK5 direct IHC stains. The infiltrating tumor cell nests of basal cell carcinoma are highlighted with direct CK5 IHC. A and B, low magnification (2X); C and D, high magnification (20X). Arrow, tumor cell nests; Arrow head, eccrine glands.



**Figure 3D.** CK5 direct IHC stain of nodular and infiltrating basal cell carcinoma. A and C, HE stains; B and D, CK5 direct IHC stains. The infiltrating tumor cell nests of basal cell carcinoma are highlighted with CK5 direct IHC stain. A, B, C and D, high magnification (20X). Arrow, tumor cell nests; Arrow head, inflammatory cells.

**A****B**

**Figure 4A.** CK5 direct IHC stain of squamous cell carcinoma. A, HE stain; B, CK5 direct IHC stain. Normal skin epidermal squamous cells, hair follicles, sebaceous glands and squamous cell carcinoma are highlighted with CK5 direct IHC, low magnification (2X).



**Figure 4B.** CK5 direct IHC stain of squamous cell carcinoma. A and C, HE stains; B and D, CK5 direct IHC stains. The infiltrating tumor cell nests of squamous cell carcinoma are highlighted with CK5 direct IHC. A, B, C and D, high magnification (20X). Arrow, tumor cell nests; Arrow head, inflammatory cells.

# Conclusions

1. This one-step CK5 direct IHC method is a simple to use; and it is ultra-fast comparing with current IHC methods. The procedure is easy to learn and simple to operate. The whole procedure can be finished within 10 min, that makes it possible to be used intraoperatively during MMS without delaying surgery.
2. The one-step CK5 direct IHC does not need expensive machine to operate and it can even be done at room temperature.
3. Our results have shown that this simple one-step CK5 direct IHC can accurately identify and map basal cell and squamous cell carcinoma on frozen section skin tissues during MMS with minimal background stains.
4. This one-step CK5 direct IHC is a potentially useful tool for MMS treatment of infiltrating type basal cell carcinoma and poorly differentiated squamous cell carcinoma.

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