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Pathology Section

# Utility of Prospective Step Sections followed by Reverse Embedding Technique in Increasing Diagnostic Accuracy of Skin Biopsies

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## ABSTRACT

**Introduction:** Small skin biopsies offer a cosmetic advantage to the patient but may provide limited information for making a diagnosis. Non specific and overlapping microscopic features often seen on superficial histopathology sections contribute to this challenge. In such cases, the use of step deeper and reverse embedding (re-embedding) sections has utility in improving diagnostic accuracy in dermatopathology practice.

**Aim:** To examine the use of prospective step sectioning and reverse embedding in skin biopsies to improve diagnosis.

**Materials and Methods:** This prospective, cross-sectional study included 200 consecutive skin biopsies received in the Department of Pathology, BGS Global Institute of Medical Sciences, Bengaluru, Karnataka, India, over an eight-month period from June 2022 to January 2023. Only skin biopsies smaller than 5 mm were included, while large punch biopsies (larger than 5 mm) were excluded. For each sample, a superficial section, step deeper section, and reverse embed section were taken. The pathologist reviewed the microscopic findings and rendered a diagnosis on the first slide. The other two slides were then reviewed, and the information provided by slides 2 and 3 was categorised as either no new information, additional information to make a diagnosis, or a change in diagnosis. Any change in diagnosis based on the information from slides 2 and 3 was noted and analysed.

**Results:** Out of the 200 skin biopsies studied, 32 cases (16%) were non diagnostic on the first slide. Step deeper sections helped in making a diagnosis in 16 (8%) cases, and reverse embedding aided in the diagnosis of 9 (4.5%) cases. For the remaining seven cases where no additional information was obtained even after deeper and reverse embed sectioning, a descriptive report was provided. In eight (4%) cases out of the 200 biopsies where a diagnosis was made on the first slide, deeper/reverse embedding led to a change in diagnosis. Thus, deeper sectioning and reverse embedding improved diagnostic accuracy in 33 cases out of the total 200 skin biopsies studied (16.5%).

**Conclusion:** This study highlights the utility of step deeper and reverse embed (re-embedded) sections in increasing diagnostic accuracy in small skin biopsies. Therefore, implementation of standardised protocol for studying multiple sections of small skin biopsies before rendering a diagnosis can significantly reduce diagnostic errors and aid in providing appropriate treatment to patients.

**Keywords:** Histopathology, Re-embedding, Reverse embedding, Small skin biopsies, Step deeper

## INTRODUCTION

Dermatopathology is largely concerned with the ability to classify diseases into categories that will help predict clinically important decisions, such as treatment and prognosis [1]. Based on the clinical scenario, various techniques of skin biopsies are available. To ensure good representation of the lesion and hence better interpretation, the right lesion and the right technique should be employed for performing the biopsy [2]. Proper orientation of the specimen is of utmost importance during paraffin embedding, and embedding very small skin biopsies requires expertise. Incorrect embedding of tissue samples leads to improper diagnosis and may require re-embedding or reverse embedding of the tissue [3]. While it is possible to orient the tissue after wax infiltration, studies have shown that it is often more convenient to orient the tissue during the grossing process itself. Agar-based pre-embedding techniques have been tried for skin biopsies to avoid mal-orientation-related problems. This allows the pathologist to control how tissues will be arranged in the final paraffin block [4,5]. Most dermatopathology specimens are very small biopsies, and therefore additional sections are often taken in the histopathology laboratory to obtain maximum information for an accurate diagnosis [6]. Depending on the need, different types of sections, such as serial sections and step deeper sections, can be taken [7]. While serial sections provide more diagnostic information, step sections are particularly useful when no useful information is obtained from superficial sections or when sections are required for special stains and other ancillary tests [8]. When no additional information is possible even with deeper sections, the re-embedding (reverse embedding) technique can be performed. However, standardised protocols for processing dermatopathology specimens in the laboratory are lacking, and studies related to the utility of deeper and reverse embedding sections in dermatopathology are very scarce in the literature [6,9]. The aim of this study was to examine the use of prospective step sectioning and reverse embedding in skin biopsies to improve diagnosis.

## MATERIALS AND METHODS

This prospective, cross-sectional study comprised 200 consecutive skin biopsies received in the Department of Pathology, BGS Global Institute of Medical Sciences, Bengaluru, Karnataka, India, over a period of 8 months from June 2022 to January 2023. The Institutional Ethical Committee (IEC) approval was taken with IEC number is BGSGIMS/IEC/APP/Feb/2023/05.

**Inclusion criteria:** Consecutive skin biopsies smaller than 5 mm, received in the Department of Pathology, were included in the study.

**Exclusion criteria:** Large punch biopsies (>5 mm) and excision biopsies were excluded from the study.

**Sample size estimation:** The sample size was calculated using the reference article by Sangeetha RS et al., [7]. The following formula for proportion was used:

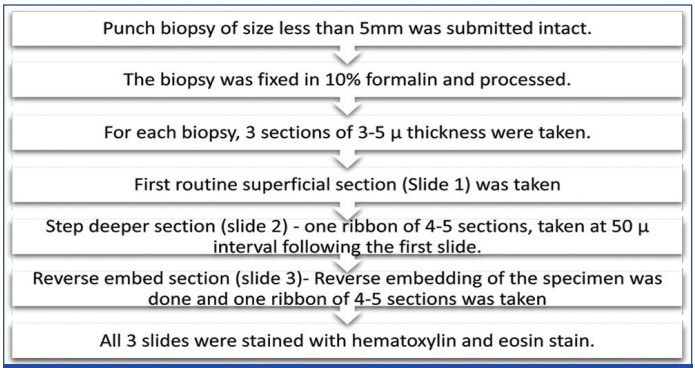
$$n=\frac{Z^2_{\alpha}pq}{d^2}$$

where 'p' is the proportion of skin biopsies that require the step section method for the final diagnosis, q=1-p, 'z' is the value for the α level, α is the significance level, and 'd' is the precision level. In this case, 'p' was taken as 23.3% from the reference journal with a precision level of 6% and a significance level of 5% [3].

$n=\frac{1.96^2\times0.233\times0.767}{0.06^2}=191$, which can be approximated to 200.

Hence, the required sample size for conducting the study was 200.

**Methodology:** Patient's clinical details, such as age, sex, site of the lesion, and clinical diagnosis, were documented. A few intervening sections between slides 1 and 2 were set aside, unstained, in case special stains/immunohistochemistry were required. The pathologist reviewed the microscopic findings and rendered a diagnosis on the first slide. Subsequently, the other 2 slides were reviewed by the pathologist, and the information provided by slides 2 and 3 was classified as follows: no new information, additional information to make a diagnosis, or change in diagnosis. Any change in diagnosis based on the information from slides 2 and 3 was noted and analysed [Table/Fig-1].



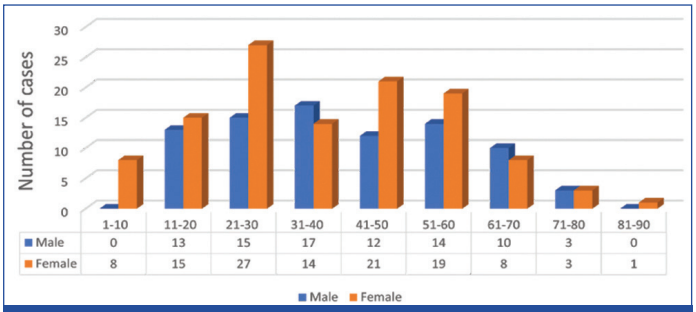

**[Table/Fig-1]:** Showing flow chart of the process.

## STATISTICAL ANALYSIS

The collected data was entered and analysed using Microsoft Excel. The data was expressed in percentages (%).

## RESULTS

A total of 200 consecutive skin biopsies were studied. The median age at presentation was 36.5 years, and the range was from 2 to 90 years. The male-to-female patient ratio was 0.7:1 [Table/Fig-2].




**[Table/Fig-2]:** Age range and sex distribution of skin cases included in the study (n=200).

The most common site for skin biopsies was the lower limb, followed by the upper limb and head and neck [Table/Fig-3]. The most common clinical finding was a plaque (36% of cases), followed by a patch (24.5%) and a papule (21.5%).

| Site | Number (%) |
|---|---|
| Head and neck | 28 (14) |
| Chest | 9 (4.5) |
| Back | 27 (13.5) |
| Abdomen | 14 (7) |
| Upper limb | 45 (22.5) |
| Lower limb | 77 (38.5) |
| Total | 200 (100) |

**[Table/Fig-3]:** Site distribution of skin biopsies (n=200).

Out of the 200 skin biopsies studied, 32 cases (16%) were non diagnostic on the first slide. Step deeper sections were helpful in making a diagnosis in 16 (8%) cases [Table/Fig-4]. Reverse embed sections provided additional information to make a diagnosis in nine cases [Table/Fig-5].

| Case no. | Age (years) | Sex | Site | Slide 1 (Superficial) | Slide 2 (Step deeper section) |
|---|---|---|---|---|---|
| 3 | 46 | F | Neck | Benign spindle cell lesion | Neurofibroma |
| 7 | 21 | F | Back | Lichenoid reaction pattern | Lichen striatus |
| 13 | 39 | M | Abdomen | Non specific findings | Lichen planus pigmentosus |
| 16 | 23 | F | Cheek | Psoriasiform epidermal hyperplasia | Inflammatory linear verrucous epidermal nevus |
| 21 | 12 | M | Leg | Non specific findings | Pustular psoriasis |
| 27 | 50 | F | Leg | Bulla was not identified | Subcorneal pustular dermatoses |
| 28 | 19 | F | Thigh | Lichenoid reaction | Lichen planus |
| 42 | 55 | M | Abdomen | Ill formed granuloma with perivascular inflammation | Leprosy-borderline tuberculoid |
| 79 | 72 | F | Back | Benign spindle cell lesion | Dermatofibroma |
| 81 | 60 | F | Leg | Subcorneal pustule with psoriasiform hyperplasia | Subcorneal pustular dermatosis |
| 110 | 32 | M | Leg | Mild perivascular mixed inflammation | Leucocytoclastic vasculitis |
| 147 | 28 | F | Scalp | Spongiosis present. Fungal elements were absent | Tinea incognito |
| 168 | 43 | F | Arm | Non specific findings | Reactive perforating collagenosis |
| 170 | 58 | M | Thigh | Basal vacuolar degeneration with mild perivascular inflammation | Lichen amyloidosis |
| 181 | 25 | F | Leg | Crateriform epidermis with no molluscum bodies | Molluscum contagiosum |
| 183 | 21 | F | Arm | Psoriasiform dermatoses | Psoriasis |

**[Table/Fig-4]:** Cases in which there was additional information available on step deeper section to make a diagnosis (n=16).

| Case no. | Age (years) | Sex | Site | Slide 1 (Superficial) | Slide 3 (Reverse embed section) |
|---|---|---|---|---|---|
| 1 | 56 | M | Leg | Ulceration with dense mixed inflammation | Pyoderma gangrenosum |
| 34 | 40 | F | Forearm | Increased pigmentation of basal layer | Dowling diego |
| 53 | 18 | M | Foot | Non specific changes with mild hyperkeratosis | Acrokeratosis verruciformis |

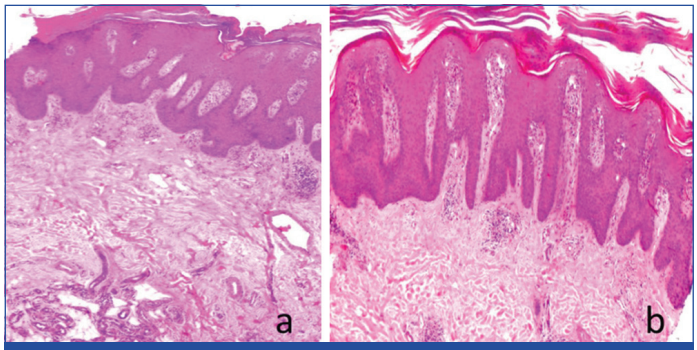
| | | | | | |
|---|---|---|---|---|---|
| 61 | 67 | F | Foot | Ulceration with dense mixed inflammation and granulation tissue | Squamous cell carcinoma |
| 69 | 28 | F | Leg | Lichenoid reaction | Lichen planus |
| 99 | 29 | F | Abdomen | Mild perivascular lymphocytic inflammation | Lichen planus |
| 106 | 36 | F | Forehead | Lichenoid reaction | Discoid lupus erythematosus |
| 192 | 45 | F | Chest | Mild perivascular inflammation | Small vessel neutrophilic vasculitis |
| 190 | 28 | F | Leg | Epidermis absent, dense inflammation in dermis | Kyrle disease |

**[Table/Fig-5]:** Cases in which there was additional information available on reverse embed section to make a diagnosis (n=9).

The initial section of a case of a 67-year-old female with a non healing ulcer over the foot (case 61) and a strong clinical suspicion of malignancy revealed only ulceration with dense mixed inflammation and granulation tissue formation. There was no definitive evidence of malignancy. Step deeper sections revealed changes in the adjacent epidermis that were limited to reactive cellular changes. Reverse embed section showed full-thickness epidermal dysplasia, keratin perls, and atypical mitoses with areas of invasion, which helped in making a diagnosis of squamous cell carcinoma.

The initial histopathology sections of a 28-year-old female patient with hyperpigmented papules over bilateral lower limbs (case 69) showed mild basal vacuolar degeneration and lymphocytic infiltration with scattered melanophages in the dermis. Step deeper sections revealed a more intense interface lichenoid reaction. However, on reverse embed, increased inflammation with shoulder hypergranulosis and civatte bodies were seen, and the case was diagnosed as lichen planus.

One case of psoriasis on initial sections showed features of psoriasiform epidermal hyperplasia with mild spongiosis and superficial lympho-histiocytic infiltrate (case 183). On step deeper sections, parakeratosis with Munro microabscesses and supra-papillary thinning were evident and more pronounced on reverse embed section [Table/Fig-6].

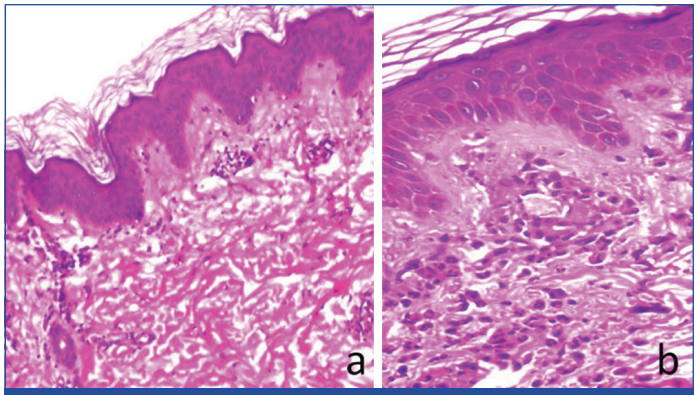



**[Table/Fig-6]:** Case of psoriasis with features more pronounced on reverse embed (b) (H&E stain, X100).

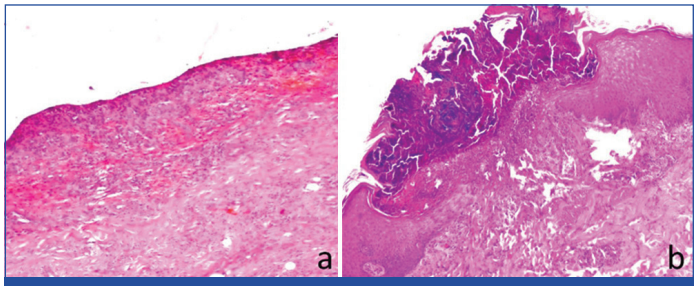
Case 192 on initial and step deeper sections showed mild perivascular neutrophilic infiltrate [Table/Fig-7]. On reverse embed section, fibrinoid necrosis of the blood vessel wall with leucocytoclasis was noted, and a diagnosis of small vessel vasculitis was made.

Epidermis was not present in the initial and step deeper sections of case 190, but reverse embed section revealed epidermal invagination filled with keratin, degenerated cellular and inflammatory debris, and dense mixed inflammation in the dermis. A diagnosis of Kyrle disease was made [Table/Fig-8].

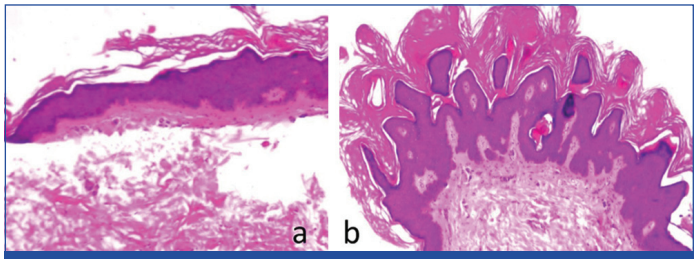
Case 53, with clinical suspicion of Acrokeratosis verruciformis, showed mild hyperkeratosis in the epidermis on the first section as well as on the deeper step. On reverse embedding, hyperkeratosis, regular acanthosis, and low papillomatosis were observed, with no parakeratosis or epidermal vacuolisation consistent with Acrokeratosis verruciformis [Table/Fig-9].




**[Table/Fig-7]:** Small vessel vasculitis, initial level (a) showed only mild perivascular inflammation and was later diagnosed on reverse embed (b) (H&E, X400).




**[Table/Fig-8]:** Initial and step deeper section (a) showed dense mixed inflammation in the dermis. Epidermis was not seen (H&E stain, X400) Reverse embed section (b) showed overlying keratotic plug with transepidermal elimination of amorphous cellular debri in an atrophic-appearing epidermis with dense mixed inflammation in the dermis (H&E stain, X100).



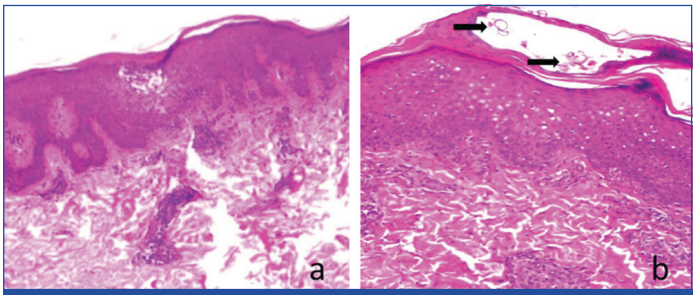

**[Table/Fig-9]:** Acrokeratosis verruciformis, initial section (a); diagnosed on reverse embed section (b) (H&E stain, X100).

Out of the 200 biopsies, eight (4%) cases had a change in diagnosis when deeper/reverse embedding was performed after rendering an initial diagnosis on the first slide [Table/Fig-10].

| Case no. | Age (years) | Sex | Site | Slide 1 (superficial) | Slide 2 (Step deeper section) | Slide 3 (Reverse embed section) |
|---|---|---|---|---|---|---|
| 9 | 43 | M | Back | Ashy dermatoses | Ashy dermatoses | Lichen planus |
| 11 | 60 | F | Leg | Actinic keratosis | Actinic keratosis | Basal cell carcinoma |
| 20 | 58 | M | Chest | Prurigo simplex | Prurigo simplex | Scabies |
| 26 | 18 | M | Abdomen | Lentigo | Lentigo | Nevus |
| 62 | 19 | F | Back | Foreign body reaction | Foreign body reaction | Epidermal inclusion cyst |
| 96 | 22 | F | Leg | Nummular dermatitis | Psoriasis | Psoriasis |
| 100 | 63 | F | Leg | Discoid lupus erythematosus | Lichen planus | Lichen planus |
| 104 | 16 | F | Forearm | Non specific changes | Urticarial vasculitis | Urticarial vasculitis |

**[Table/Fig-10]:** Cases in which there was Change in diagnosis (n=8).

In the case of clinical suspicion of Prurigo simplex (case 20), the initial section showed mild acanthosis, parakeratosis, and spongiosis with a mild perivascular lymphocytic inflammatory infiltrate. These findings were more pronounced in the deeper step section. However, reverse embed sections revealed the presence of pink pigtail-like structures and scybala in the stratum corneum [Table/Fig-11]. A diagnosis of scabies was made.




**[Table/Fig-11]:** A case of suspected prurigo simplex showed epidermis with mild spongiosis, spongiotic versiculation, along with mild superficial and deep lymphocytic infiltrate within the dermis in the first and step deeper section (a). On reverse embed, Pink pigtails (egg fragments-arrows) and excreta of mite noted in the stratum corneum revealing the diagnosis of scabies (b) (H&E, X100).

In the case of clinical suspicion of nevus (case 26), mild uniform elongation of the rete with increased melanin in the basal layer was observed. The initial section and deeper step section did not reveal nevus cells. However, on reverse embedding, proliferation of nevus cells was noted at the dermo-epidermal junction and in the dermis. In case number 62, the initial and deeper step sections showed only dense mixed inflammation with granulation tissue and foreign body giant cell reaction to some keratin fibers. No cyst was noted. However, on reverse embedding, a cyst wall lined by stratified squamous epithelium was discernible.

For the remaining seven cases [Table/Fig-12], where no additional information was available even after deeper and reverse embedding, a descriptive report was provided. Hence, deeper sectioning and reverse embedding helped improve diagnostic accuracy in 33 cases (16.5%). Additionally, in 36 cases (18%) out of the total 200 biopsies, where a diagnosis was possible on the first slide, deeper and reverse embed sections helped clarify and confirm the diagnosis. Overall, 69 cases out of 200 cases (34.5%) required additional sections (deeper section and reverse embed section) to confirm or clarify an initial diagnosis.

| Case no. | Age (years) | Sex | Site | Clinical diagnosis | Slide 1 and 2 (Superficial and step deeper) | Slide 3 (Reverse embed section) |
|---|---|---|---|---|---|---|
| 2 | 32 | M | Back | Vasculitis | Mild perivascular lymphocytic infiltration | Mild perivascular lymphocytic infiltration |
| 10 | 19 | M | Upper limb | Vitiligo | No pigment alteration | No pigment alteration |
| 22 | 20 | M | Abdomen | Psoriasis | Mild non specific changes | Mild non specific changes |
| 40 | 57 | F | Back | Discoid lupus erythematosus | Epidermal atrophy | Epidermal atrophy with mild perivascular inflammation |
| 59 | 65 | F | Lower limb | Psoriasis | Mild perivascular inflammation | Acanthosis with congested blood vessels and mild perivascular inflammation |
| 78 | 26 | M | Lower limb | Lichen planus | Non specific changes | Non specific changes |
| 94 | 70 | M | Lower limb | Leprosy | Scarce perivascular lymphocytic inflammation | Scarce perivascular lymphocytic inflammation |

**[Table/Fig-12]:** Cases where no additional information was available on step deeper/reverse embed section, to make a diagnosis (n=7).

## DISCUSSION

Small skin biopsies have taken a central place in dermatology practice due to cosmetic concerns [1]. However, this decrease in specimen size poses challenges for histopathologists in making accurate diagnoses. Inability to bisect these tiny specimens during orientation and embedding can further decrease diagnostic accuracy. To address this, deeper sections are often ordered in dermatopathology to improve diagnostic sensitivity and accuracy. Studies indicate that deeper sections provide a more accurate diagnosis in approximately one-third (33%) of skin biopsy specimens [6]. In a study by Sangeetha RS et al., 23.3% of skin biopsies required deeper sections for an accurate diagnosis [7]. In present study, 34.5% of cases required deeper sections or reverse embedding for a final diagnosis.

Based on the need, different types of sections can be taken [7]. In serial sectioning, sections are collected from the very first cut and a continuous ribbon of sections are placed on multiple slides [9]. Step sections on the other hand are taken at periodic levels through the block. The request is made for every nth section for a total of 'n' sections [10]. Step sections are useful as intervening unstained slides can be kept for ancillary tests such as special stains wherever required [11]. Normally, deeper levels are requested following review of the first slide by the pathologist. This often leads to delay in turnaround time. In some histopathology laboratories, step deeper sections are also prepared prospectively and submitted to the pathologist along with the original slide for review [12].

Of the 200 skin biopsies studied, 32 cases (16%) were non diagnostic on the first slide. Step deeper sections were helpful in making a diagnosis in 16 (8%) cases and reverse embedding helped in the diagnosis of 9 (4.5%) cases [Table/Fig-4,5]. Thorough examination of multiple sections should be done to avoid diagnostic errors. Study of multiple sections is particularly useful in cases of malignancy. In case 61, the initial section revealed only ulceration with dense mixed inflammation and granulation tissue formation. Step deeper sections revealed reactive cellular changes. Reverse embed section was required to make a diagnosis of squamous cell carcinoma. Ashy dermatoses in active phase present with lichenoid reaction at the dermo-epidermal junction with basal vacuolar degeneration reminiscent of Lichen planus [1]. Case 69 showed mild basal vacuolar degeneration and lymphocytic infiltration with scattered melanophages in the dermis. Step deeper section revealed more intense interface lichenoid reaction. However, reverse embed sections helped in making the diagnosis of Lichen planus. Cutaneous small vessel vasculitisor leukocytoclastic vasculitis is characterised by perivascular neutrophilic infiltration with occasional lymphocytes, endothelial cell swelling and fibrinoid necrosis of blood vessel wall. Karyorrhexis (leucocytoclasis) of WBCs noted [13]. Case 192 on initial and step deeper section showed non specific changes such as mild perivascular neutrophilic infiltrate [Table/Fig-7]. On reverse embed section, fibrinoid necrosis of blood vessel wall with leucocytoclasis was noted.

Literature has shown that studying multiple deeper sections, especially in small biopsies, can significantly reduce diagnostic errors [6,12,14-16]. However, other studies have indicated a tendency to request extensive and unwarranted deeper sections in search of a diagnosis [17,18]. There is currently no standardised protocol for determining the number of deeper sections necessary for

an accurate diagnosis in dermatopathology practice [15,19]. Additionally, small skin biopsies pose challenges for specimen orientation during processing, leading to difficulties in achieving correct orientation without crushing artifacts [20,21]. This can result in misdiagnosis, where reverse embedding becomes helpful. In present study, step deeper and reverse embed sections assisted in changing the diagnosis for eight cases [Table/Fig-10]. For a clinically suspected case of prurigo simplex, initial and step deeper sections revealed spongiosis and mild perivascular inflammation, while reverse embed section showed the presence of pink pigtail-like structures and scybala in the stratum corneum [Table/Fig-11]. This aided in diagnosing scabies and initiating appropriate therapy, emphasising the importance of reverse embed sections. Similarly, in case number 62, an epidermal inclusion cyst was identified only on reverse embed, while the initial superficial and step deeper sections showed dense mixed inflammation with granulation tissue and foreign body giant cell reaction to some keratin fibers.

A skin biopsy of approximately 3 mm in size is considered the smallest size that can provide sufficient information for an accurate diagnosis while minimising scarring to the patient [22]. It is equally important to reach the subcutis during the biopsy, as the subcutaneous tissue has a rich supply of small capillaries that aid in faster healing with minimal scarring [23]. Biopsies taken from areas with relatively avascular dermis as their base are more prone to causing slough at the biopsy site and have an increased risk of secondary infection [2,24]. In this study, the majority of cases (96%) were non neoplastic, as excisional biopsies, the preferred technique for evaluating neoplastic lesions, were excluded. Out of the eight neoplastic cases, only four were malignant, and step deeper and reverse embed techniques were helpful in achieving accurate diagnoses in these cases. Similar findings have been observed in various other studies [2,15,25-27].

A study conducted by Kattel G et al., demonstrated a statistically significant reduction in turnaround time and increased sensitivity when using prospective deeper sections for small skin biopsies [6]. Therefore, the slight increase in cost associated with using prospective step deeper sections and reverse embed sections in routine dermatopathology practice may be offset by the production of reports with superior diagnostic accuracy within a shorter period [12].

### Limitation(s)

The utility of retrospective step deeper and reverse embed sections in improving diagnostic accuracy may be limited by the tendency to increase turnaround time. On the other hand, prospective deeper and reverse embed sections may not be necessary for all cases and could potentially impose an unwanted economic burden. Studies could be conducted to evaluate the cost-benefit ratio of prospective versus retrospective deeper sectioning before establishing a protocol for processing dermatopathology specimens in the laboratory.

## CONCLUSION(S)

This study highlights the utility of prospective step deeper and reverse embed sections in increasing the diagnostic accuracy of skin biopsies. While prospective step deeper and reverse embed sectioning have obvious advantages, such as increased diagnostic accuracy, they also come with some drawbacks, including a slight increase in cost, increased workload for technicians, and the requirement for more storage space for slides. However, histotechnicians find it easier and faster to cut and section specimens prospectively rather than wasting time retrieving archived blocks and preparing them for cutting and staining.

### Acknowledgement

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