# Optimal Level of Sections for Definite Diagnosis in Transbronchial Biopsy and Pleural Biopsy Specimens

**Panthep Suttinont, M.D., Usanee Nakphong, M.D.**

*Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.*

## ABSTRACT

**Objective:** To determine optimal level of serial section in transbronchial and pleural biopsy that yield maximal definite diagnosis.

**Methods:** A cross sectional study of 118 transbronchial biopsy and pleural biopsy specimens submitted with serial sectioning in 3 levels were performed. Specimens of 1 mm. in diameter were serially cut and slides at levels I, II, III (120, 240 and 360 μm.) from initial exposure of tissue in paraffin blocks were studied, and specimens of 2-3 mm. in diameter were cut at levels I, II, III (0, 120 and 240 μm.) after tissues in paraffin blocks were trimmed to expose maximal diameter. Comparisons of diagnoses of each level were done.

**Results:** The percentages of definite diagnoses were 90.2, 96.1 and 93.2 in sections of level I, II and III, respectively. Chronic granulomatous lesions were found in section level II more than other levels, but there was no statistical significance. (P value 0.131, Chi-Square test)

**Conclusion:** Transbronchial and pleural biopsy specimens should be cut deep to level II, one slide for hematoxylin-eosin staining and 3 unstained slides for further investigation.

**Keywords:** Optimal level of section, transbronchial biopsy, pleural biopsy

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Transbronchial biopsy is extremely useful and accurate in the diagnosis of lung cancer and infection especially in an immunocompromised host and certain diffuse lung diseases such as sarcoidosis. It was developed by Anderson and colleague utilizing a blunt, flexible forceps passed through a rigid bronchoscope without fluoroscopic guidance. There is evidence that transbronchial lung biopsy via either the rigid or flexible bronchoscope is a safe procedure with good diagnostic yield in diffuse lung disease.[1-6]

Pleural biopsy is a well established technique for investigation of pleural diseases and effusion.[7] Pleural biopsy findings may include granuloma in pleural tuberculosis, metastatic carcinoma, primary pleural tumor and malignant lymphoma.[8]

For biopsy pieces about 2 mm. in diameter, Sheppard suggested that they should be cut at 30-50 μm. intervals to give three sections of each level in one slide. Three spare unstained slides are taken at each of the three levels for further special staining.[9]

In another study[10] the diagnostic significance of the step sections in nondiagnostic transbronchial biopsies obtained from patients with diffuse or multiple lung diseases were evaluated. Step sections were especially useful for the detection of epithelioid granulomas and tumor tissues in patients with sarcoidosis and lymphangitic carcinomatosis. They prepared multiple additional serial sections from the paraffin-embedded transbronchial lung biopsy specimens as follows; fifty sections 3 μm. in thickness each were cut serially from the blocks and every five sections (step sections) were stained with hematoxylin and eosin (H&E). The remaining sections were preserved and used for special staining if necessary. The result was that useful findings were obtained by step sections is 30/112 cases (26.8%). The routine sectioning of transbronchial biopsy and pleural biopsy specimens at the Department of Pathology, Siriraj Hospital, Mahidol University were performed as follows; if specimen size was about 1 mm, it was cut for 4 serial sections in 1 slide, each section was 2-3 μm

*Correspondence to: Panthep Suttinont*
*E-mail: sipst@mahidol.ac.th*

in thickness and was stained with H&E. If specimen size was about 2-3 mm, it was cut until the maximum diameter was revealed then 4 serial sections in 1 slide with H&E staining were prepared. If step sectioning was needed in a problem case, the paraffin block was trimmed, so some tissue was partially lost. Thus diagnostic yield in this intermittent step sectioning was not as good as serial step sectioning, but serial sectioning for many levels had more cost.

The study of the optimal level of sections for definite diagnosis in transbronchial biopsy and pleural biopsy has not yet been established at Siriraj Hospital. This study was performed on new cases of transbronchial and pleural biopsy to find out the optimal level for the maximum yield for definite diagnosis and without needing to do serial sectioning of the whole tissue.

## MATERIALS AND METHODS

The study samples were 118 cases performed on transbronchial biopsy and pleural biopsy specimens at the Department of Pathology, Siriraj Hospital, Mahidol University during September 2007 to February 2008, by the criteria below. The study was approved by the Siriraj Ethics Committee. Certificate of Approval no Si 326/2007.

**Inclusion criteria (cases)**

- 118 cases from transbronchial biopsy and pleural biopsy.
- Adequate material for study.

**Exclusion criteria (cases)**

- Other biopsy procedure of lung tissue.
- Inadequate material for study.

**Sample Size**

Estimated sample size in our study was 118 cases, calculated on the prevalence in a previous study[10] that 26.8% more diagnostic yield was obtained from step sectioning of nondiagnostic transbronchial biopsy in diffuse lung disease.

**Study procedure**

- This study was performed on transbronchial biopsy and pleural biopsy cases at the Department of Pathology, Siriraj Hospital, Mahidol University.
- Each slide contained 4 continuous sections, each section was 2 μm in thickness.
- If tissue biopsy was about 1 mm. in diameter, 3 microscopic slides of serial sectioning at levels I, II, III were taken at 120, 240 and 360 μm., from the first exposure of tissue at the paraffin block surface, (slide nos. 15, 30 and 45) and were stained with H&E, 6 unstained slides at 216, 224, 232, 336, 344 and 352 μm., (slide nos. 27, 28, 29, 42, 43 and 44) were spared for special staining.
- If tissue biopsy was about 2-3 mm., in diameter. It was cut until the maximum diameter of tissue was revealed. Then 3 microscopic slides of serial sectioning at levels I, II, III that were 0, 120 and 240 μm., from the maximum diameter plane respectively (slide nos. 1, 15 and 30) were stained with H&E, 6 unstained slides at 96, 104, 112, 216, 224 and 232 μm. (slide nos. 12, 13, 14, 27, 28 and 29) were spared for special staining.
- In routine work, they used only slides at level I of both categories for H&E staining.

**Data analysis**

Fisher's exact test was used to compare outcomes between levels of each diagnostic category.

## RESULTS

The total numbers of the study group were 118 cases of 102 transbronchial biopsy and 16 pleural biopsy specimens. The definite diagnosis can be drawn in H&E slides at level I - 107 cases (90.2%), level II - 114 cases (96.1%) and level III - 110 cases (93.2%) respectively. All specimens that were diagnosed in level I also had the same result in level II.

There was no significant difference in the diagnostic yield of all levels (p-value = 0.131). When compared between level I and II, level II and III, level I and III, the p-values were 0.086, 0.568 and 0.360 respectively.

In 118 cases, diagnoses divided into 4 groups; 51 cases were malignancy; 12 cases were chronic granulomatous inflammation; 26 cases were non-specific chronic inflammation; and 29 cases were negative. In the malignancy group, 47 cases (92.16%), 49 cases (96.07%) and 48 cases (94.12%) were diagnosed in

**TABLE 1.** Percentage of diagnosis in 4 groups.

| Diagnosis | Level I | | Level II | | Level III | |
|---|---|---|---|---|---|---|
| | A | B | A | B | A | B |
| Malignancy | 47 (92.16%) | 4 (7.84%) | 49 (96.07%) | 2 (3.93%) | 48 (94.12%) | 3 (5.88%) |
| Chronic granulomatous inflammation | 7 (58.33%) | 5 (42.67%) | 11 (91.67%) | 1 (8.33%) | 8 (66.67%) | 4 (33.33%) |
| Non-specific Chronic inflammation | 24 (92.31%) | 2 (7.69%) | 25 (96.15%) | 1 (3.85%) | 25 (96.15%) | 1 (3.85%) |
| Negative | 29 (100%) | 0 | 29 (100%) | 0 | 29 (100%) | 0 |
| Total | 118 | | 118 | | 118 | |

A = definite diagnosis; B = no definite diagnosis

No significant difference when comparing diagnostic yield in all levels of each group by Fisher's exact test. In chronic granulomatous inflammation group for level I and II, level II and III, p-value are 0.155 and 0.317 respectively.

serial sections level I, II and III, respectively. In the chronic granulomatous inflammation group, 7 cases (58.33%), 11 cases (91.67%) and 8 cases (66.67%) were diagnosed in serial sections level I, II and III, respectively. In the non-specific chronic inflammation group, 24 cases (92.3%), 25 cases (96.15%) and 25 cases (96.15%) were diagnosed in serial sections level I, II and III, respectively. These results are summarized in Table 1.

## DISCUSSION

Transbronchial and pleural biopsy specimens are small and lesions may be cut through when trimming paraffin block. Serial sectioning will avoid partial tissue loss from trimming and yield more definite diagnosis but more glass slides will be used than cutting at the chosen definite level. Many studies confirm the significance of step sectioning in nondiagnostic biopsy of the first cut, but there has been no recommendation for the optimal level in practical use cutting. Our study used a method which corresponds to procedures in routine work which provides slides of 4 serial sections with 2 μm., in thickness each. Tissue in level II that was obtained at a level of 240 μm., from the initial exposure of the tissue in a paraffin block of specimen size about 1 mm., and level II at 120 μm., from the maximum diameter of tissue in a paraffin block of specimen size about 2-3 mm., revealed the most diagnostic yield.

Level II is one step deeper than the level used in routine cutting of both 1 mm., and 2-3 mm., tissue sizes. When we try step sectioning of the whole tissue, level II is nearer to the central portion of the tissue more than level I. This should be reasonable for more diagnostic yield especially in small lesions with a random distribution such as chronic granulomatous inflammation. Although there is no statistical significance, it may be useful if applied for routine work.

## CONCLUSION

Serial sectioning avoids partial tissue loss from trimming. In this study, a deep tissue cut to level II was recommended for one H&E slide and three or more unstained slides prior to the H&E slide should be prepared for further investigation, proposed for maximal definite diagnosis.

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