

## Detection of Fungi in Tissue Sections Using Peroxidase-labeled Lectins

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**SUMMARY:** A battery of 8 peroxidase-labeled lectins was tested on sections of paraffin-embedded human tissues to determine which lectin could be used in the microscopic diagnosis of fungi. The lectin histochemistry was more clearly in identifying *Candida*, *Aspergillus*, *Mucor*, and *Cryptococcus* than fluorescein isothiocyanate-labeled lectins because these fungi were fluorescent in sections of paraffin-embedded tissue under ultraviolet illumination. Five lectins, from *Ricinus communis* (RCA-120), *Lens culinaris* (LCA), *Ulex europaeus* (UEA-1), *Canavalin eniformis* (ConA), and *Triticum vulgare* (WGA), were found to react with fungal pathogens commonly encountered in nosocomial infections. Three lectins, from *Arachis hypogaea* (PNA), *Phaseolus vulgaris* (PHA-E), and *Dolichos biflorus* (DAB) were stained weakly or not at all with fungi.

### INTRODUCTION

Lectins were probably first described by Stillmark in 1888 and have been defined recently as sugar-binding proteins or glycoproteins of non-immune origin, which agglutinate cells and/or precipitate glycoconjugates having saccharides of appropriate complementarity.<sup>6)</sup> Cellular glycoconjugates consist of carbohydrate-rich molecules, including glycoproteins and glycolipids, that are widely distributed intracellularly, on the cell surface coat, and in the extracellular matrix. Currently lectins conjugated to a visulant are used as histochemical reagents to identify and localize specific carbohydrate residues in normal and abnormal tissues, and from the application of lectins as very simple and selective tools for the detection of carbohydrate groups. In the present study the author used 8 different lectins to analyze fungi. The investigation was undertaken as an extension of my previous work.<sup>14)</sup>

### MATERIALS AND METHODS

The specimens studied were obtained from biopsy and autopsy materials at the Nagasaki University Hospital. Confirmation of fungi morphology was provided with periodic acid Schiff (PAS), and Grocott's methenamine silver (GMS). Hematoxylin-and-eosin stained sections were used for examination using a transmitted light fluorescence microscope (Zeiss, FRG). Fungi were identified by light and fluorescence microscope as *Candida*, *Aspergillus*, *Mucor* and *Cryptococcus*. The tissues were fixed in 10% formaldehyde, and routinely embedded in paraffin. Tissues were cut at 4 micron and stained with peroxidase-labeled lectins such as *Triticum vulgare* (WGA), *Ulex europaeus* (UEA-1), *Arachis hypogaea* (PNA), *Ricinus communis* (RCA-120), *Dolichos biflorus* (DAB), *Phaseolus vulgaris* (PHA-E), *Lens culinaris* (LCA), and *Canavalin eniformis* (ConA).

The steps involved in peroxidase-labeled lectins for fungi are the following: (1) Deparaffinize

and hydrate to distilled water. (2) Treat with bath phosphate buffer saline (PBS) pH 7.2 for 10 minutes. (3) Treat with peroxidase-labeled lectins for one hour in moisture chamber at room temperature. (4) Treat with bath PBS, 3 changes of 5 minutes each. (5) Wash in distilled water. (6) DAB/H<sub>2</sub>O<sub>2</sub> substrate 5-30 minutes. (7) Wash in distilled water. (8) Perform nuclear stain in hematoxylin. (9) Wash in running water. (10) Dehydrate, Clear and mount. The peroxidase-labeled lectins were purchased from Honnen Oil Company, Tokyo, Japan as a solution.

## RESULTS

All paraffin sections included in this study contained fungi that were identified by light microscopy examination of hematoxylin-and-eosin and were also routinely stained with periodic acid Schiff (PAS) and Grocott's methenamine-silver slides. These fungi have been found to autofluorescence in routine hematoxylin-and-eosin sections when exposed to ultraviolet illumination. The methods were useful in identifying *Candida*, *Aspergillus*, *Mucor* and *Cryptococcus*. *Candida* (Fig. 1), *Aspergillus* (Fig. 2), *Mucor* (Fig. 3), and *Cryptococcus* (Fig. 4) were stained with *R communis*, *L culinaris*, *U europaeus*, *C ensiformis* and *T vulgare*. These fungi stained weakly or not at all with *A hypaea*, *P vulgaris*, and *D biflorus*.

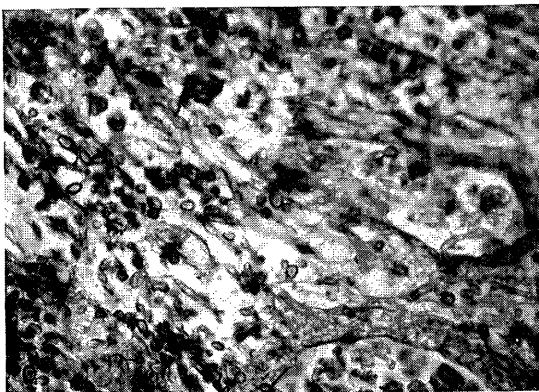


Fig. 1. *Candida* in lung reacted with peroxidase lectin (*Lens culinaris*, ×400).

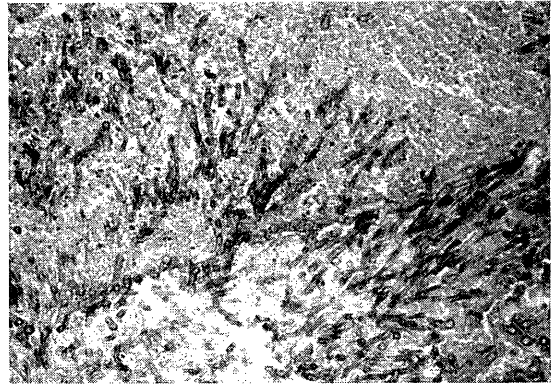


Fig. 2. *Aspergillus* in lung reacted with peroxidase lectin (*Canavalin ensiformis*, ×200).



Fig. 3. *Mucor* in lung reacted with peroxidase lectin (*Ulex europaeus*, ×200).

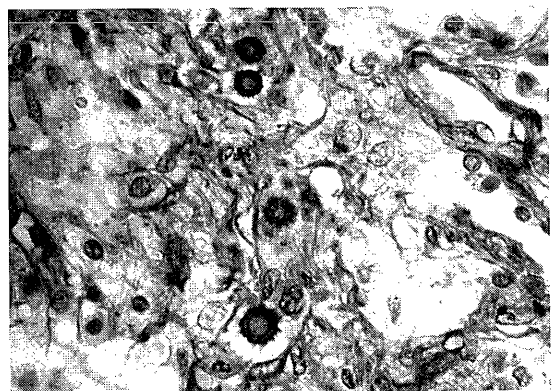


Fig. 4. *Cryptococcus* in lung with peroxidase lectin (*Ricinus communis*, ×400).

## DISCUSSION

Lectins are proteins or glycoproteins of non-immune origin that can bind carbohydrate residues in a very specific way. Their specificity in recognizing carbohydrates is higher than obtained with other histochemical methods, thus, lectins can be used as probes to study glycoconjugate expression in the normal and disease tissues.<sup>6)</sup> The cell wall of most fungi contains glycolipids, glycoproteins, lipopolysaccharides, and other complex glycoconjugates. Several investigator reported that fluorescein isothiocyanate (FITC)-labeled lectins could be used for morphologic diagnosis of fungal infection in routinely processed tissue sections.<sup>5, 15)</sup> On the other hand, most fungi are fluorescent in hematoxylin-and-eosin stained sections of paraffin-embedded tissue under ultraviolet illumination.<sup>1~4, 7, 8)</sup> In my experience, *Candida*, *Aspergillus*, and mature *Rhinosporidium seeberi* are autofluorescent in unstained sections of paraffin-embedded tissue with ultraviolet illumination.<sup>9, 10)</sup> Moreover, these fungi in unstained sections are less fluorescent than in hematoxylin-and-eosin stained paraffin-embedded tissue with ultraviolet illumination.<sup>12, 13)</sup> Some fungi are autofluorescence in paraffin-embedded tissue under ultraviolet illumination, thus, I wonder what part fungal autofluorescence plays in the observed reaction of fungi with fluorescein isothiocyanate-labeled lectins in ultraviolet light. However, peroxidase-labeled lectins can be used to detect glycoconjugate in fungi in sections of paraffin-embedded tissue.

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