

Localization of the major house dust mite allergen *Der p I* in the body of *Dermatophagoides pteronyssinus* by ImmunoStain®

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The house dust mite *Dermatophagoides pteronyssinus* is a major source of inhalant allergens [1] and a major allergen, *Der p I*, is excreted in the faecal particles [2]. Mite allergens have been detected in frozen sections by means of immunofluorescence [3,4]. We report a sensitive non-isotopic method using enzyme labelled antibodies for immunolocalization of *Der p I* in mites embedded in paraffin wax.

Mites were harvested from active cultures, fixed in Clarke's solution for 15 min, dehydrated in absolute alcohol followed by xylene and then embedded in paraffin wax. Sections (5 µm) were cut using a conventional microtome. The slides were dewaxed and endogenous peroxidase activity was blocked with hydrogen peroxide following the ImmunoStain® kit (Euro/DPC, Witney, U.K.) procedure. Polyclonal, monospecific sheep anti-*Der p I* (1:1000) was added to the sections. The specimens were then washed prior to the addition of biotinylated donkey anti-sheep IgG (1:500). After another wash, streptavidin labelled horse-radish peroxidase was added to each section. All incubation steps were performed for 30 min at room temperature in a humidified container. The sections were washed once more, followed by the addition of 3-amino-9-ethylcarbazole (AEC) chromogen with peroxidase substrate for 15 min to produce a red reaction product. Non-immunoreactive regions were counterstained with Mayer's haematoxylin. Control sections prepared using non-immune sheep serum demonstrated that there was no endogenous peroxidase activity, nor any non-specific staining due to the other reagents.

The modification of the ImmunoStain® streptavidin/biotin immunoperoxidase method for staining histologi-

cal preparations of *D. pteronyssinus* bodies, showed that the most immunoreactive components were located in the faecal pellets, mouth and hind-gut tissues (Fig. 1). Weak, non-specific interactions were sometimes associated with other areas of the mite and may be attributed either to diffusion of the antigen during processing or to the coprophagous behaviour of the house-dust mite. However, with the methods we have used the possibility that some *Der p I* is released through the salivary ducts behind the buccal cavity cannot be ignored.

In conclusion, we have used a peroxidase immunohistochemical method to provide a coloured instead of a fluorescent endpoint. This allowed a permanent visualization of *Der p I* immunoreactive regions simultaneously with counterstained non-reactive areas. Instead of producing frozen sections, mites were embedded in paraffin wax for better morphology and effective destruction of endogenous peroxidase activity.

References

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Fig. 1. Thin section of *D. pteronyssinus* magnified 600 times after immuno-staining. *Der p I* is concentrated in the faecal pellet, the hind gut and the mouth.

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