Government of Canada / Gouvernement du Canada
Canadian Forestry Service / Service canadien des forêts

ISSN 0826-0532

1987

TECHNICAL NOTE

Forest Pest Management Institute

No. 8 JUNE 1987 Laboratory Methods

THE HIGHLIGHTING OF NUCLEAR POLYHEDROSIS VIRUS INCLUSION BODIES EMBEDDED IN THICK SECTIONS USING NAPHTHALENE BLACK 12B

It is difficult to observe nuclear polyhedrosis virus inclusion bodies (IBs) in thick (0.5 µm) sections of partially purified samples of virus embedded in Araldite. Toluidine blue (0.5%) with 1% borax is usually applied to thick sections for general staining of cellular material prior to thin sectioning for electron microscopic examination. However, Toluidine blue does not stain IBs differentially, which may result in the best area for thin sectioning being missed altogether, or could delay the processing of the sample if several different areas are chosen and sectioned. We have found that staining with Naphthalene Black 12B alleviates these problems.

Inclusion bodies are extracted from heavily infected insect larvae by first macerating the larvae in distilled water using a hand-held tissue grinder. This macerate is filtered through four layers of cheesecloth to remove large pieces of insect material. Inclusion bodies are separated out of the suspension by alternate centrifugation for 15 min at 164 g and 2638 g respectively using a Sorvall HB-4 rotor. The resulting pellet contains most of the IBs and a small amount of insect cellular debris. An aliquot of this partially purified sample of virus is resuspended in 2 mL of distilled water, placed in a conical beem capsule and centrifuged for 15 min at 2638 g to form a solid pellet. Further processing of this viral material takes place in the conical beem capsule.

The material is fixed overnight at 4°C in 5% glutaraldehyde in 0.05M sodium cacodylate buffer with 0.01M calcium chloride and 2% sucrose at pH 7.3. Fresh fixative is added and the material is centrifuged again in a Sorvall SS-3 with a SS-34 rotor at 1912 g for 30 minutes. Following two washes in 0.05M sodium cacodylate with 10% sucrose, the pellet is post-fixed in 1% osmium tetroxide with 0.05M sodium cacodylate and 4% sucrose at 4°C and pH 7.3 for 2 hours, and at room temperature for 2 hours, centrifuging for the last 30 minutes. The material is washed three times for 30 minutes each with distilled water in the centrifuge. Samples that do not pellet are embedded in agar (Glaurert 1978). The material is stained, en bloc, in 2.5% uranyl acetate overnight at 60°C. After dehydration in graded alcohols, the virus is embedded in Araldite.

Samples are thick sectioned (0.5 µm) using a glass knife on a Reichert Ultracut ultramicrotome. The thick sections are then stained for 15 minutes in a solution of 1.5% Napthalene Black 12B in a 35:65 mixture of Glacial acetic acid and distilled water, respectively, rinsed in distilled water and air dried. Stained sections are examined under oil.

Naphthalene Black 12B has been used to stain IBs for enumeration (Wigley 1980). Similarly, in our work inclusion bodies stain light blue-black while few other cell organelles take the stain

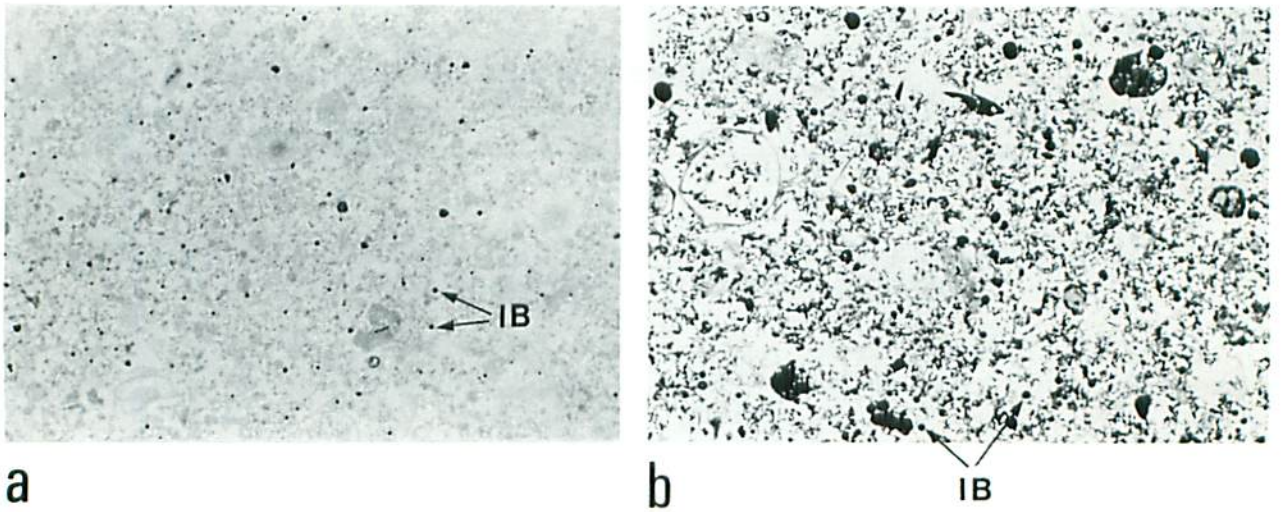

Fig. 1. Thick section of partially purified IBs stained with Naphthalene Black 12B (a), and Toluidine Blue (b). IB = inclusion bodies. X294.

making it easier to identify inclusion bodies (Fig. 1a). There is a faint background staining with Naphthalene Black 12B but this does not interfere with the detection of IBs. On the other hand, Toluidine blue (de Martino et al. 1968) is taken up by all cellular material making it very difficult to locate areas containing IBs for sectioning (Fig. 1b). Similar results are obtained when searching for IBs of a granulosis virus, although these are difficult to observe because of their small size. This stain does not provide any advantages when applied to sections of infected tissue: virus in tissue is usually detected initially by the pathology of the cells, which is more easily determined by the standard Toluidine blue stain.

In conclusion, we recommend that Naphthalene Black 12B be used to stain thick sections containing IBs, especially for partially purified samples and low level infections to increase efficiency in locating areas for thin sectioning.

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References

de Martino, C.; Natali, P.G.; Bruni, C.B.; Accini, L. 1968. Influence of plastic embedding media on staining and morphology of lipid bodies. *Histochemie* 16:350-360.

Glaurert, A.M. 1978. Fixation, dehydration and embedding of biological specimens. *in* Practical Methods In Electron Microscopy. Vol. 3(1). (ed. A. Glaurert). North-Holland Press, Amsterdam.

Wigley, P.J. 1980. Counting microorganisms. *in* Microbial Control Of Insect Pests. (J. Kalmakoff and J. Longworth eds). New Zealand Dept. of Scientific and Industrial Research DSIR Bull. 228. Wellington, New Zealand.