D-35: A simplified technique for the identification of the immature stages of *An.culicifacies*, the major malaria vector in Sri Lanka

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Vector surveillance is an important prerequisite for the planning and evaluation of any malaria vector control measure. Vector surveillance programmes now rely mainly on the measurements of the adult mosquito density. The available conventional taxonomic methods for the identification of immature stages of vector mosquitoes from those of other mosquito species are tedious. This is because such methods require the mounting of larval stages on the slides and observation under microscope, or the rearing of larval stages to obtain adults. The objective of this study was to develop a simple DNA probe technique to detect the immature stages of *An.culicifacies* in the field.

Larvae and pupae of *An. culicifacies*, *An. tesellatus*, *An. subpictus*, *Cx. quinquefasciatus*. *Ae. aegypti* were placed in duplicate on nitrocellulose (N/C) filter membrane lying on Whatman 3MM paper saturated with 10% sodium dodecyl sulphate (SDS). These mosquito samples were squashed onto the filter membrane using a glass plate by applying firm downward pressure.

The nitrocellulose filters were then placed on Whatman paper saturated with a solution of 0.5 M NaOH / 1.5 M NaCl. After 5 min, the N/C filters were transferred to a Whatman paper saturated with a solution of 0.5 M Tris / 1.5 M NaCl (pH 7.0). The air dried N/C filter was then baked for 2 h at 80°C. The prephybridized filters were then hybridized separately with ^{32}P labelled Rp217 and Rp234 DNA probes.

The filters were washed under stringent conditions (0.1 x SSC, 0.5% SDS at 50°C for 2 min) and autoradiographed for 2 h at -70°C with Kodak XAR-5 film.

The squash blot hybridization of mosquito samples with biotinlabelled probes was also carried out using the blotting procedure described above. The hybridization signals were visualized using a colorimetic detection system with a colour development time of 1 h.

Radiolabelled (^{32}P) and biotinlabelled Rp217 and Rp234 probes were used to identify the immature stages of *An. culicifacies* from those of other mosquito species. A strong positive hybridization signal was given by all immature stages (larvae and pupae) of *An. culicifacies* while those of other mosquito species gave a negative hybridization signal under high stringent filter washing conditions, even though the filters were developed only for a very short time (2 h exposure for the radiolabelled probes and 1 h of colour development for biotinlabelled probes

The mosquito squash blot technique enables a single investigator to simultaneously process a large number of mosquito samples of immature stages collected from the field. Furthermore, each immature stage of mosquito can be analysed without any loss of sample.

Due to limitations imposed by the use of radioisotopes, the biotinylated probes are more suitable than radiolabelled probes for the identification of *An. culicifacies* immature stages in the field.