A-16: A simplified non-radioactive DNA probe technique for the field detection of sibling species A of the *Anopheles culicifacies* complex

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Three cloned highly repetitive DNA sequences Rp36, Rp217 and Rp234, have been used as ^{32}P labelled probes to detect the sibling species A of the *Anopheles culicifacies* complex. The identification is carried out using dot - blot hybridization assays with single mosquito DNA extracts diluted 200 - fold. The preparation of DNA samples and the requirement of a radioisotope for labelling and the associated risks involved in handling storage and disposal of radioisotopes limits its field use. Therefore, to overcome these problems, the feasibility of using a non-radioactive biotinylated labelling and detection system was studied. It was found that this technique takes less time and could be used with all three DNA probes in similar dot-blot hybridization assays. However, the technique required 50-fold dilution of the single mosquito DNA sample for detection of species A. This assay was simplified further by the use of blots prepared from squashed mosquito heads which avoids the separate extraction of DNA and also facilitates a number of mosquitoes to be processed quickly. In addition, it allows several field analyses to be carried out on the same mosquito specimen. It was found that Rp217 DNA probe was ideal to be used in this assay. Thus, a simplified, more attractive and safe method for field use, has been developed for the detection of species A of *An. culicifacies*.