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Short Report

Infectivity of malaria vector mosquitoes: correlation of positivity between ELISA and PCR–ELISA tests

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Studies on the infectivity of malaria vector mosquitoes using the enzyme-linked immunosorbent assay (ELISA) described by WIRTZ *et al.* (1987) have been carried out worldwide for several years. SOMBOON *et al.* (1993) reported false-positive results for the ELISA associated with bovine and swine blood. In order to avoid these false-positive results it is advisable either to use only the anterior part of the mosquito (head and thorax) which, normally, is not contaminated by the ingested animal blood (WIRTZ *et al.*, 1987), or to confirm the ELISA result by another method such as polymerase chain reaction (PCR).

We have carried out the PCR–ELISA to confirm the detection of human malaria parasites in mosquitoes already recorded as positive by ELISA alone. Thirty two such mosquitoes were tested, belonging to different species of the genus *Anopheles* and collected during field trips to different areas of the Amazonia Region (Pará, Amapá, Rondônia and Roraima States). The same number of mosquitoes known to be negative for human malaria parasites was also tested.

The source of *Plasmodium* DNA was the same material used for the ELISA test [triturated mosquitoes—head and thorax ground in a blocking buffer containing 0·05% nonidet P-40 (WIRTZ *et al.*, 1987)], either 20 µL spotted on a glass fibre membrane (GFM) prepared for PCR, using 1/8 of the spot as DNA source directly into the PCR mixture as described by WARHURST *et al.* (1991), or extracted as follows: 40 µL of ELISA solution was centrifuged for 10 min at 22 500 ***g***, the pellet was lysed by adding 25 µL of lysis buffer and incubating at 65°C for 30 min, afterwards adding potassium acetate and placing on ice for at least 60 min. The isolated DNA (obtained by ethanol precipitation) was dissolved in 15 µL of TE-buffer containing ribonuclease (WILSON *et al.*, 1998). The DNA was amplified as described by MACHADO *et al.* (1998) using primer sequence, concentrations and reaction conditions indicated by OLIVEIRA *et al.* (1995). For the identification of the human malaria parasites we used the liquid-phase, non-isotopic hybridization ELISA technique, following the protocol of OLIVEIRA *et al.* (1995). For negative controls we used distilled water, male anopheline and culicine mosquitoes, and human DNA. Positive controls included strain K1 of *P. falciparum*, and mosquitoes experimentally infected with *P. falciparum* and *P. vivax*.

Our PCR results confirmed the ELISA test results for all positive and all negative mosquitoes, and in 5 (15·6%) of the positive mosquitoes (3 *An. albitarsis* and 2 *An. darlingi*) the PCR–ELISA technique detected other species of human *Plasmodium* that were not found by the ELISA test alone (Table). The DNA source was obtained only by DNA extraction, which means that the GFM technique is not applicable for this type of material. These results indicate that the PCR–ELISA is more sensitive than a simple ELISA test which, however, still remains a very good and useful tool for testing mosquito infectivity.

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Table. Comparison between ELISA and PCR-ELISA results for human malaria parasites in *Anopheles* mosquitoes from several areas of the Amazon region in Brazil

Species	State	ELISA	PCR–ELISA
An. (Nys.) albitarsis	Roraima	*Pv/Pf*	*Pv/Pf/Pm*
An. (Nys.) albitarsis	Roraima	*Pv/Pf*	*Pv/Pf*
An. (Nys.) albitarsis	Roraima	*Pv/Pf*	*Pv/Pf*
An. (Nys.) albitarsis	Roraima	*Pv/Pf*	*Pv/Pf*
An. (Nys.) braziliensis	Roraima	*Pv/Pf*	*Pv/Pf*
An. (Nys.) nuneztovari	Roraima	*Pv/Pf*	*Pv/Pf*
An. (Nys.) darlingi	Rondônia	*Pf*	*Pf/Pm*
An. (Nys.) darlingi	Rondônia	*Pf*	*Pf/Pv*
An. (Nys.) albitarsis	Amapá	*Pm*	*Pf/Pm*
An. (Nys.) albitarsis	Amapá	*Pm*	*Pf/Pm*
An. (Nys.) albitarsis	Amapá	*Pf*	*Pf*
An. (Nys.) albitarsis	Amapá	*Pv*	*Pv*
An. (Nys.) albitarsis	Amapá	*Pv*	*Pv*
An. (Nys.) albitarsis	Amapá	*Pm*	*Pm*
An. (Nys.) albitarsis	Amapá	*Pv*	*Pv*
An. (Nys.) braziliensis	Amapá	*Pv*	*Pv*
An. (Nys.) darlingi	Amapá	*Pf*	*Pf*
An. (Nys.) darlingi	Amapá	*Pm*	*Pm*
An. (Nys.) aquasalis	Pará	*Pf*	*Pf*
An. (Nys.) aquasalis	Pará	*Pf*	*Pf*
An. (Nys.) aquasalis	Pará	*Pv*	*Pv*
An. (Nys.) darlingi	Pará	*Pv*	*Pv*
An. (Nys.) darlingi	Pará	*Pv*	*Pv*
An. (Nys.) darlingi	Pará	*Pf*	*Pf*
An. (Nys.) darlingi	Pará	*Pf*	*Pf*
An. (Nys.) nuneztovari	Pará	*Pf/Pv*	*Pf/Pv*
An. (Nys.) nuneztovari	Pará	*Pf*	*Pf*
An. (Nys.) nuneztovari	Pará	*Pf*	*Pf*
An. (Nys.) nuneztovari	Pará	*Pf*	*Pf*
An. (Nys.) nuneztovari	Pará	*Pv*	*Pv*
An. (Nys.) nuneztovari	Pará	*Pm*	*Pm*
An. (Nys.) nuneztovari	Pará	*Pm*	*Pm*

Pv, *Plasmodium vivax*; *Pf*, *P. falciparum*; *Pm*, *P. malariae*.

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