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Touchdown-touchup nested PCR for low-copy gene detection of benzimidazole-susceptible Wuchereria bancrofti with a Wolbachia endosymbiont imported by migrant carriers

(2011) *Experimental Parasitology*, 127 (2), pp. 559-568. Cited 1 time.

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Abstract
A novel, sensitive and specific touchdown-touchup nested PCR (TNPCR) technique based on two useful molecular markers, a Wuchereria bancrofti β-tubulin gene involved in benzimidazole susceptibility and a Wolbachia ftsZ gene involved in cell division, was developed to simultaneously detect the parasite W. bancrofti (W1) with its Wolbachia endosymbiont (W2) from both microfilaremic and post-treatment samples of at-risk migrant carriers infected with geographical W. bancrofti isolates. The detection and characterization of authentically low-copy gene-derived amplicons revealed no false positive identifications in amicrofilaremia with or without antigenemia. The W1-TNPCR was 100-fold more sensitive than the W2-TNPCR regardless of the microfilarial DNA isolation method and compared well with the thick blood film and membrane filtration techniques. These locus-specific TNPCRs could also detect Wolbachia-carrying W. bancrofti genotype in addition to a link to benzimidazole sensitivity among those with unknown infection origins that exhibited microfilaremia responsiveness against treatment with diethylcarbamazine plus albendazole. These TNPCR methods can augment the results of microscopic detection of the parasite because these methods enhance DNA isolation and PCR amplification capabilities. © 2010 Elsevier Inc.

Author Keywords
β-tubulin; Benzimidazole susceptibility; DNA isolation; FtsZ; PCR amplification; Touchdown-touchup nested PCR; Wolbachia; Wuchereria bancrofti

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Document Type: Article
Source: Scopus