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(54) **FLAVIVIRUS DETECTION AND QUANTIFICATION ASSAY**

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(51) **Int. Cl.**[7] **C12Q 1/68**; C12P 19/34; C07H 21/04

(52) **U.S. Cl.** **433/6**; 435/91.1; 435/91.2; 536/23.1; 536/24.3

(58) **Field of Search** 435/6, 91.1, 91.2; 536/23.1, 24.3

(56) **References Cited**

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(List continued on next page.)

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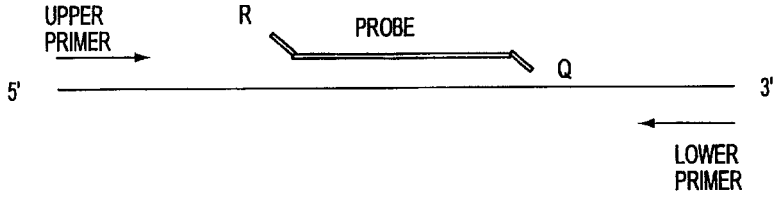
(57) **ABSTRACT**

A fluorescent DNA probes specific to the conserved terminal 3'-noncoding region (nucleotides 10653–10678) of dengue virus and a pair of flanking primers are designed to formulate a dengue specific fluorogenic polymerase chain reaction (PCR). Optimal assay conditions with zero background are disclosed which permit the detection of low levels of dengue virus from clinical specimens. Dengue virus isolates from different geographic regions can be universally detected and identified by the fluorogenic RT-PCR assay. Moreover, the assay is specific for dengue 2 virus and does not recognize other related flaviviruses, including dengue serotypes Louis encephalitis, yellow fever, and Kunjin viruses. The) 3, and 4, Japanese encephalitis, St. assay also efficiently detected immunocomplexed dengue viruses. The fluorogenic RT-PCR assay readily detected viremia in sera collected from individuals ill with dengue fever.

21 Claims, 8 Drawing Sheets

DENGUE SEROTYPE DETECTION:	SEROTYPE-SPECIFIC UPPER PRIMERS:	INTERNAL FLUOROGENIC PROBE	LOWER PRIMERS (ANTI-SENSE, OR RT PRIMER)
DENGUE 1	DV1.U (SEQ ID NO:15) 5'-ACA-CCA-GGG-GAA-GCT-GTA-TCC-TGG-3'	DV.P1 (SEQ ID NO: 1) 5'-CTG-TCT-CCT-CAG-CAT-CAT-TCC-AGG-CA-3'	DV.L1 (SEQ ID NO:2) 5'-CAT-TCC-ATT-TTC-TGG-CGT-TCT-3'
DENGUE 2	DV2.U (SEQ ID NO:3) 5'-AAG-GTG-AGA-TGA-AGC-TGT-AGT-CTC-3'		
DENGUE 3	DV3.U (SEQ ID NO:16) 5'AGC-ACT-GAG-GGA-AGC-TGT-ACC-TCC-3'		
DENGUE 4	DV4.U (SEQ ID NO:17) 5'-AAG-CCA-GGA-GGA-AGC-TGT-ACT-CCT-3'	DV.P2 (SEQ ID NO:9) 5'-CTG-TCT-CTG-CAA-CAT-CAA-TCC-AGG-CA-3'	DV.L2 (SEQ ID NO:10) 5'-CAA-TCC-ATC-TTG-CGG-CGC-TCT-3'