

Quantitative Detection of *Tropheryma whipplei* DNA by Real-Time PCR

Over the past decade, PCR-based methodologies have been introduced to complement or even replace histopathologic study of biopsy specimens for the diagnosis of Whipple's disease (12). However, positive PCR results have been reported on testing small-bowel and saliva specimens from asymptomatic patients (3, 4, 11). Although these results have not been independently confirmed (8), they have nonetheless led to a poor predictive diagnostic value for these house-made PCR assays that lack of controls. In response to this concern, we evaluated a quantitative real-time PCR (LightCycler; Roche, Mannheim, Germany) combining rapid cycling with fluorescence-based identification of PCR products in glass capillaries (13, 14) for the diagnosis of Whipple's disease with two pairs of primers targeting different genes. We determined a diagnostic cutoff value based on evaluating the detection level by a titration of cells infected with *Tropheryma whipplei*, the agent of the disease (6, 10) and negative controls (uninfected cells). A positive PCR result was defined by a fluorescent signal equivalent to that derived from at least 10 copies of standard control DNA (Fig. 1). The assay was evaluated on seven frozen duodenal biopsies, one frozen lymph node biopsy and one frozen cardiac valve obtained from nine patients with histologically proven Whipple's disease (9) and a control group composed of 150 duodenal biopsy specimens, 20 lymph node biopsy samples, and 100 saliva specimens from people with no suspicion of Whipple's disease.

We used primers *tws3f* and *tws4r* to target a 489-bp fragment of the 16S-23S ribosomal DNA intergenic spacer (ITS) (5) and primers *TWRPOB.F* and *TWRPOB.R* to target a 650-bp fragment of the β -subunit of the RNA polymerase gene (*rpoB*) (1). Either 25 mg of tissue or 1 ml of aspirate from each sample was used for DNA extraction, as previously described (3). Mixes were prepared by following the manufacturer's instructions (FastStart DNA Master SYBR Green; Roche). The LightCy-

TABLE 1. Number of DNA copies detected for nine patients with Whipple's disease

Patient/sex/age ^a	Sample	Number of copies detected	
		ITS	<i>rpoB</i>
1/M/48	Lymph node tissue	150,000	170,000
2/M/62	Duodenal biopsy specimen	30,000	58,000
3/M/46	Duodenal biopsy specimen	300	120
4/F/33	Duodenal biopsy specimen	100	100
5/M/57	Duodenal biopsy specimen	20,000	15,000
6/F/71	Duodenal biopsy specimen	10,000	6,500
7/M/61	Duodenal biopsy specimen	500	500
8/M/66	Duodenal biopsy specimen	900	1,000
9/M/63	Cardiac valve specimen	900	1,200

^a M, male; F, female. Ages are given in years.

cler PCR result, with both primer pairs, was positive for each of the nine patients with Whipple's disease, with each yielding similar quantitative results (Table 1). All the 150 duodenal biopsy samples, 20 lymph node biopsy samples, and 100 saliva samples from the control group failed to yield a significant PCR product signal.

The epidemiology of Whipple's disease remains unclear. The bacterium may well be present in the environment (7); thus humans may be regularly exposed to, or colonized by, but not necessarily infected with, *T. whipplei*. In such circumstances, the potential ability of quantitative PCRs to differentiate between environmental contamination or low-level colonization and the higher concentration of bacteria associated with clinical manifestation becomes apparent. Thus, introduction of an evaluated and commercially available

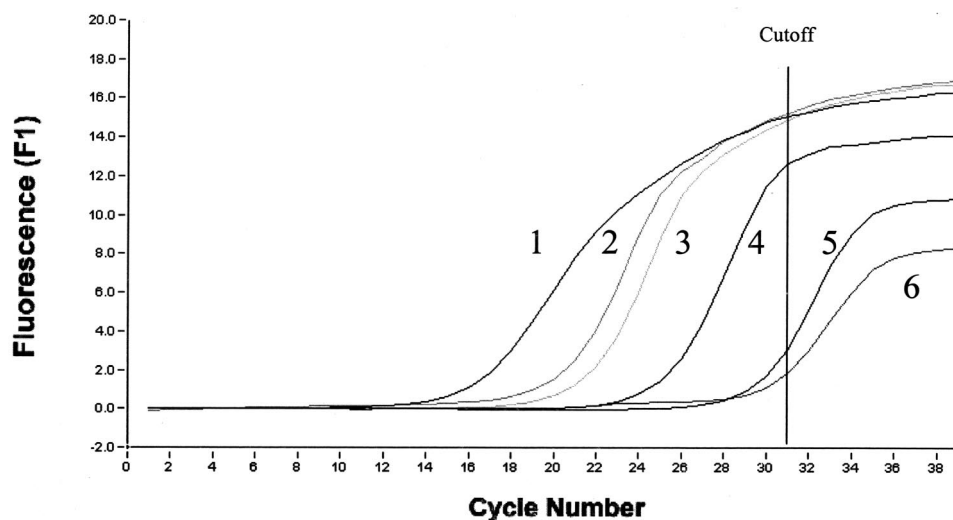


FIG. 1. Detection of *T. whipplei* DNA using the LightCycler technique performed with 16S-23S rDNA(ITS)-derived primers. The amplification of copies of the DNA of 10^4 copies from DNA standard *T. whipplei* (1), positive duodenal biopsy from patient 2 (2), positive duodenal biopsy from patient 6 (3), 10 copies from DNA standard *T. whipplei* (4), a negative duodenal biopsy from control group (5), uninfected cells using as negative control (6).

LightCycler PCR procedure seems well adapted to the diagnosis of Whipple's disease.

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