TITLE: A READY TO USE, DUPLEX qPCR FOR DIFFERENTIAL DIAGNOSTIC OF *BORDETELLA PERTUSSIS* USING REAGENTS PRODUCED IN BRAZIL

AUTHORS: ZAHRA, N. M; MORELLO, L.G.; COSTA, A.D.T.

INSTITUTION: INSTITUTO DE BIOLOGIA MOLECULAR DO PARANÁ (CURITIBA-BRAZIL).

ABSTRACT

Whooping cough is an infectious disease caused by bacteria of the *bordetella* genus. Currently, culture from deep nasal swab is the gold-standard diagnostic test. *Bordetella* spp. a fastidious species, thus culturing becomes a time-consuming test, hampering the correct treatment. Here, we present a multiplex diagnostic test based on Real-Time PCR (qPCR) to detect, discriminate and identify the main species of whooping cough-causing bacteria*: B. pertussis, B. parapertussis and B. holmesii*. The protocol was optimized in a ready-to-use (gelified) format, eliminating the need for freezing temperatures during transportation and storage. A human genomic target (18S rRNA or RNaseP) was used as internal reaction control. We compared imported reagents to the ones produced by the Instituto de Biologia Molecular do Paraná (IBMP), aiming to decrease the need of imported products and reduce costs. Our results show that IBMP's reagents presented similar or better efficiencies than the imported ones. Both reaction formats (liquid or gelified) exhibited the same sensitivity: 0.02 bacterial genome equivalents per microliter. Amplicons from limiting dilutions were confirmed by sequencing. The test was validated using positive clinical samples, and the resulting algorithm yielded a more sensitive analysis of the samples, allowing for a more accurate diagnostic. No nonspecific reactions were observed when different species of human pathogenic bacteria and fungi were tested with the qPCR. The use of a Brazilian qPCR diagnostic kit grants purchasing agility, without compromising accuracy and sensitivity, contributing to the proper clinical management of patients while strengthening the national industry.

Keywords: *Bordetella pertussis*, whooping cough, Real Time PCR, molecular diagnosis, ready to use.

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