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Scientific letter

Performance of 2 automated real time PCR methods for the detection of *Bordetella pertussis* and *Bordetella parapertussis*

Rendimiento de 2 técnicas de PCR en tiempo real para la detección de Bordetella pertussis *y* Bordetella parapertussis

The aetiological agent of pertussis is *B. pertussis,* but *B. parapertussis* and *B. holmesii* can cause very similar clinical manifestations.[1] *B. bronchiseptica* infection is rare, clinically distinct and affects debilitated patients.[2] The most commonly used targets for the diagnosis of *Bordetella* spp. by PCR have been the IS*481* and IS*1001* sequences. IS*481* is present in *B. pertussis* and *B. holmesii*, and may also be present in *B. bronchiseptica.* IS*1001* is found in *B. parapertussis* and occasionally in *B. bronchiseptica*.[3–5] One way to distinguish species is based on the use of specific primers for the promoter region of the pertussis toxin gene *(ptxA-pr),* which is specific to *B. pertussis*.[3,5,6] An alternative is the *B. pertussis* porin gene *(BPTD_0837)*.[4] The *BP283* gene has also been used to identify *B. pertussis*.[7] Numerous kits are now available for the molecular diagnosis of pertussis. However, their interpretation can be complex due to the possibility of detecting the same sequences in different species. The aim of this study was to evaluate the performance of two real-time PCR methods, RealCycler® BORD-T (Progenie Molecular), and Simplexa™ Bordetella Direct, (DiaSorin Molecular LLC) for the diagnosis of pertussis.

Fifty nasopharyngeal swab/nasopharyngeal wash samples obtained from patients with clinical suspicion of pertussis between July 2018 and January 2020 were studied, and 44 nasopharyngeal swab samples received in February 2022 for diagnosis of SARS-CoV-2 by RT-PCR were studied as a control group. Samples were kept frozen at −80°C until studied. All samples were from epidemiological surveillance studies and were processed simultaneously with the RealCycler® BORD-T assay after nucleic acid extraction and Simplexa™ Bordetella Direct assay directly from the sample without prior extraction. The results of the two techniques were interpreted according to the manufacturers' respective recommendations.

For the 50 suspected pertussis samples, the results with RealCycler® BORD-T were: 28 (56%) *B. pertussis*, 12 (24%) *Bordetella* spp., six (12%) *B. parapertussis* or *B. bronchiseptica*, three (6%) co-infection with different *Bordetella* spp. and one (2%) negative. With Simplexa™ Bordetella Direct, the results were: 39 (78%) *B. pertussis*, six (12%) *B. parapertussis*, two (4%) co-infection by different *Bordetella* spp., two (4%) negative and one (2%) invalid due to amplification inhibition (Table 1). In 47 samples with suspicion of pertussis (94%), the two methods matched regarding the identification at the genus level or co-infection with *Bordetella* spp. In 27 (54%) of these cases the two techniques identified the species as *B. pertussis*. In 11 cases (22%) where Simplexa™ Bordetella Direct classified the result as *B. pertussis*, RealCycler® BORD-T classified it as *Bordetella* spp. In the six cases (12%) identified as *B. parapertussis* by Simplexa™ Bordetella Direct, the RealCycler® BORD-T result was either *B. parapertussis* or *B. bronchiseptica*. All 44 samples collected for SARS-CoV-2 detection were negative for *Bordetella* spp. in both techniques.

The RealCycler® BORD-T assay kit detects both IS*481* and the BP*283* region. This combination incorporates high sensitivity and

Table 1
Distribution of RealCycler® BORD-T and Simplexa™ Bordetella Direct results in terms of the total number of samples of suspected pertussis studied.

RealCycler® BORD-T				Simplexa™ Bordetella Direct			Total	
	IS*481*	IS*1001*	BP*283*		IS*481*	IS*1001*	N	%
B. pertussis	Positive	Negative	Positive	*B. pertussis*	Positive[a]	Negative[a]	27	54
Bordetella spp.	Positive	Negative	Negative	*B. pertussis*	Positive[a]	Negative[a]	11	22
B. parapertussis or *B. bronchiseptica*	Negative	Positive	Negative	*B. parapertussis*	Negative[b]	Positive[b]	6	12
[1,0]Co-infection by	Positive	Positive	Positive	[1,0]*B. pertussis* and *B.*	Positive[b]	Positive[b]	1	4
different *Bordetella* spp.	Positive	Positive	Negative	*parapertussis*	Positive[b]	Positive[b]	1	
Co-infection by different *Bordetella* spp.	Positive	Positive	Positive	*B. pertussis*	Positive	Negative	1	2
Bordetella spp.	Positive	Negative	Negative	Negative	Negative	Negative	1	2
Negative	Negative	Negative	Negative	Negative	Negative	Negative	1	2
B. pertussis	Positive	Negative	Positive	Invalid[c]	Invalid	Invalid	1	2
							50	100

The results in this table are only applicable when the internal quality specifications of each of the techniques evaluated (e.g., amplification of internal controls and/or Ct within range) are met.

[a] This combination of results from Simplexa™ Bordetella Direct would not rule out infection by *B. holmesii.*

[b] This combination of results from Simplexa™ Bordetella Direct would not rule out infection by *B. bronchiseptica.*

[c] Invalid due to amplification inhibition.

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high specificity for pertussis diagnosis. This kit also includes IS*1001* to detect *B. parapertussis*.

Simplexa™ Bordetella includes IS*481* for the detection of *B. pertussis* and IS*1001* for *B. parapertussis*.[8] Although the combination of positivity and negativity for each of these targets does not definitively exclude others such as *B. holmesii* or *B. bronchiseptica*, for practical purposes, in samples with clinical suspicion of pertussis the result could be considered probable infection by *B. pertussis* or B. *parapertussis*, as appropriate.[3] This kit has shown very good sensitivity and specificity[9] and excellent overall percent agreement values.[10]

In this study the number of samples studied is small. Furthermore, there was no gold standard to assess the sensitivity of the techniques, no control strains were available and discordant cases were not confirmed with a third alternative technique. However, the results of the two techniques matched well for the detection of *Bordetella* spp. Each technique offers its own advantages: RealCycler® BORD-T would theoretically be highly specific for *B. pertussis*, while Simplexa™ Bordetella Direct does not require nucleic acid extraction, making it a simple and rapid alternative.

Conflicts of interest

In this study the Simplexa™ Bordetella Direct (DiaSorin Molecular LLC) reagents were provided by DiaSorin Iberia S.A. Authors V.B. and E.M. are employees of DiaSorin Iberia S.A.

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