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Follow-Up of External Quality Controls for PCR-Based Diagnosis of Whooping Cough in a Hospital Laboratory Network (Renacoq) and in Other Hospital and Private Laboratories in France

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The French National Reference Centre (NRC) for Whooping Cough carried out an external quality control (QC) analysis in 2010 for the PCR diagnosis of whooping cough. The main objective of the study was to assess the impact of this QC in the participating laboratories through a repeat analysis in 2012.

Whooping cough, a highly contagious respiratory disease affecting humans (1), can be diagnosed by culture, PCR, or serology (2). PCR, and particularly real-time PCR (RT-PCR) methods, overcomes some of the limitations of culture and serology and has become the most widely used method for diagnosing *Bordetella* infection. Insertion sequences IS*481* and IS*1001* are the most frequently used targets for the detection of *B. pertussis* and *B. parapertussis*, respectively. However, the IS*481* sequence is also present in the *B. holmesii* genome (3). Moreover, *B. bronchiseptica* is causing problems for current consensus molecular diagnostic tests for pertussis, because some isolates harbor IS*481* (4) and/or IS*1001* (5). Following on the initial proficiency program (6), the French National Reference Centre (NRC) offered quality controls (QCs) to the pediatric hospital laboratory network (7) and some other hospital and private medical laboratories in 2010 (QC-2010) and 2012 (QC-2012). The main objectives were (i) to provide an overview of the PCR methods used by the laboratories and of the interpretation of the data obtained and (ii) to assess the impact of QC-2010 on the participating laboratories by repeat evaluation in 2012. Participation was voluntary, with 33 laboratories participating in QC-2010 and 35 in QC-2012. The participating laboratories were asked to complete a technical questionnaire (Table 1) to provide information about their equipment, reagents, and methods. The DNA samples of *Bordetella* isolates harboring the IS*481* or IS*1001* sequence in their genome were prepared by NRC and sent to the participating laboratories (Table 2). Each proficiency panel consisted of six specimens, each containing DNA at a concentration of 0.01 to 100.00 pg/μl or molecular biology-quality water. The participants were asked to report the positive or negative result obtained for DNA detection for each tube, together with the crossover threshold (*Ct*) value and the conclusive sentence routinely used for such situations in their analysis reports. The correct interpretation for a sample that tests positive for IS*481* or IS*1001* should be "detection of *Bordetella* species DNA." The conclusive sentence was considered incorrect if the laboratory interpreted a positive test for IS*481* as indicating that the sample corresponded to *B. pertussis* or a positive test for IS*1001* as indicating that the sample corresponded to *B. parapertussis*. The results were compiled and analyzed by NRC and then transmitted to all participants.

A summary of the technical questionnaire completed by the laboratories is presented in Table 1. In 2010 and 2012, half of the laboratories routinely used commercial kits, a third used an automated system for DNA extraction from respiratory samples, and the others were still using a manual in-house method. In 2012, two manufacturers (Roche and Cepheid) accounted for 75% of the PCR instruments used by the participating laboratories. The frequency of use of IS*1001* and IS*481* targets in combination increased from 53% in 2010 to 74% in 2012. The increase in the number of laboratories using the IS*1001* target improved the surveillance of *B. parapertussis* or, in some cases, *B. bronchiseptica* circulation in France. Only a few laboratories used additional targets and had the capacity to specifically test the presence of *B. pertussis or B. holmesii* DNA (Table 1). All except one laboratory used RT-PCR in 2010 and 2012. The proportion of laboratories that used commercial kits for the amplification step increased from 33% in 2010 to 62% in 2012. Commercial kits systematically include an internal control for validation of the extraction step and/or amplification, increasing the reproducibility of testing (8).

We analyzed the results for PCR sensitivity of the two QC exercises (Table 2). In 2010, the external QC included two negative and four positive samples. All of the participating laboratories detected the DNA in the four positive samples, regardless of the *Bordetella* species concerned. Three laboratories reported at least one false-positive result (two in a negative sample). To overcome the risk of a false-positive result, it is crucial to use a clean space for the PCR assay. The interval of the *Ct* values was between 17 and 18, regardless of the concentration of DNA and the targets used, reflecting the heterogeneity of the techniques. In 2012, the QC included one negative and five positive samples. A majority of the participants gave correct results. How-

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TABLE 1 Summary of participating laboratories responses to the technical questionnaire

Analysis yr	Extraction method (%)	Target combination (%)	Real-time apparatus type (%)[b]	No. of cycles (%)	Probe type (%)	Amplification mixture (%)	Use of extraction (%)/amplification control(s) (%)
2010 ($n = 32$[a])							
No. of answers	28	31	30	29	30	30	29
	Commercial kit (50)	IS*481*/IS*1001* (47)	LightCycler (Roche) (37)	40 (55)	Hydrolysis (73)	Commercial kit (33)	76/100
	Automated system (36)	IS*481* (44)	SmartCycler (Cepheid) (26)	45 (41)	Hybridization (17)	PCR master mix completed with oligos[c] (67)	
	In-house method (14)	IS*481*/IS*1001*/*ptx*A or *ptx*A-Pr (6)	ABI Prism (Applied Biosystems) (20)	35 (4)	SYBR Green (7)		
		IS*481*/*ptx*A (3)	Rotor-Gene (10)		Beacon (3)		
2012 ($n = 34$[a])							
No. of answers	34	34	34	34	34	34	34
	Commercial kit (52)	IS*481*/IS*1001* (71)	LightCycler (Roche) (40)	45 (59)	Hydrolysis (79)	Commercial kit (62)	84/97
	Automated system (38)	IS*481* (23)	SmartCycler (Cepheid) (35)	40 (26)	Hybridization (15)	PCR master mix completed with oligos[c] (38)	
	In-house method (13)	IS*481*/ptxA (3)	ABI Prism (Applied Biosystems) (12)	50 (15)	SYBR Green (3)		
		IS*481*/IS*1001*/*ptx*A-Pr/*recA*-h (3)	Rotor-Gene (9)		Beacon (3)		

[a] Participants using RT-PCR methodology are presented. The only laboratory using endpoint PCR method (Genoquick Bordetella Hain LifeScience kit; IS*481*-IS*1001*; Perkin Elmer apparatus) is not included in the table.
[b] LightCycler 1/2/480; SmartCycler I/II; ABI Prism 7000/7500/7900; Rotor-Gene 6000.
[c] Participants used RT-PCR mixture from different suppliers (Applied Biosystems, Roche, Eurogentec) completed with primers and probes from different suppliers.

ever, three laboratories reported at least one false-negative sample, and one laboratory reported both a false-positive and a false-negative sample. Finally, a laboratory disarrayed the tubes from the panel and reported several incorrect results. As in 2010, we found that the laboratories were using diverse amplification methods, but 91% were able to detect the lowest concentration of B. *pertussis* DNA (0.01 pg/μl).

The participants were asked to report the conclusive sentence they routinely used in their analysis reports. In 2010, among the laboratories obtaining correct results ($n = 30$), only 4 laboratories gave the correct interpretation, regardless of the target used, and 4 others gave the correct interpretation for IS*481* but an incorrect conclusion for the IS*1001* target. In 2012, the number of laboratories giving correct interpretations was higher than in 2010, contributing to an improvement in reporting: among the laboratories obtaining correct results ($n = 29$), 11 laboratories gave the correct conclusion, regardless of the target used, and 7 laboratories gave the correct interpretation for IS*481* but an incorrect conclusion for the IS*1001* target.

TABLE 2 Proficiency panel results of the QC-2010 and QC-2012 samples

Yr of analysis and code	NRC proficiency panels[a]		Results for IS*481* or **IS*1001*** detection by laboratories[b]			
	Sample	*Ct*[c]	No. of positive/total answers[d]	Mean *Ct*[e] (no. of samples)	*Ct* interval	*Ct* CV (%)
2010						
QC10-1	Water	Undet	1/33	34 (1)	NA	NA
QC10-2	*B. pertussis* 10 pg/μl	22	33/33	21 (30)	14–32	18
QC10-3	*B. parapertussis* 1pg/μl	25	18/18	25 (16)	19–36	15
QC10-4	*Water*	Undet	1/33	40 (1)	NA	NA
QC10-5	*B. pertussis* 0.1 pg/μl	28	33/33	27 (30)	21–38	13
QC10-6	*B. holmesii* 10 pg/μl	23	33/33	22 (30)	15–32	18
2012						
QC12-1	*B. holmesii* 10 pg/μl	23	34/35	22 (33)	20–28	8
QC12-2	*B. parapertussis* 0.1 pg/μl	29	24/27	31 (23)	29-41	9
QC12-3	*B. bronchiseptica* 100 pg/μl	26	32/35	26 (31)	23–36	11
QC12-4	*B. pertussis* 0.01 pg/μl	31	32/35	31 (31)	28–37	6
QC12-5	Water	Undet	2/35	39 (2)	NA	NA
QC12-6	*B. pertussis* 1 pg/μl	23	35/35	24 (34)	20–28	8

[a] *Ct*, cycle threshold; undet, undetermined.
[b] CV, coefficient of variation (ratio of the standard deviation to the mean of the *Ct* values); NA, not applicable.
[c] Average *Ct* of at least two replicates.
[d] The laboratory using endpoint PCR method ($n = 1$) is only included in the positive/total answers.
[e] Mean and interval of *Ct* calculated with the values reported by the participating laboratories.

In conclusion, the major problem identified in this study was the incorrect interpretation of results obtained by the participating laboratories, as already reported by the European surveillance network for vaccine-preventable disease (EUVAC.Net) (9). A positive result in the IS*481* assay does not constitute proof of the presence of *B. pertussis* DNA. Instead, it should be interpreted as demonstrating the presence of genetic material from *Bordetella* species. Likewise, a positive result in the IS*1001* assay does not constitute proof of the presence of *B. parapertussis* DNA, as it may, in some cases, be due to the presence of DNA from *B. bronchiseptica*. It should therefore be indicated in the analysis report, even if infrequent. This is particularly important for adolescent and adult populations. As previously shown, *B. holmesii* carriage is mostly found in teenagers and adults, at least in Europe and North America (10, 11), although it has been detected in some newborns in Romania, Chile, and Argentina (12–14). In these populations, it is important to identify the *Bordetella* species present in the samples, particularly when vaccine effectiveness or the duration of vaccine-induced immunity is determined.

External QC campaigns for the diagnosis of whooping cough by PCR should be organized regularly (ideally every 2 years) by the NRC to assess the effects of proficiency panels on laboratory performances, particularly for reporting of results and interpretation.

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REFERENCES

1. **Hegerle N, Guiso N.** 2013. Epidemiology of whooping cough & typing of *Bordetella pertussis*. Future Microbiol **8:**1391–1403. http://dx.doi.org/10.2217/fmb.13.111.
2. **Wirsing von König CH.** 2014. Pertussis diagnostics: overview and impact of immunization. Expert Rev Vaccines **13:**1167–1174. http://dx.doi.org/10.1586/14760584.2014.950237.
3. **Riffelmann M, Wirsing von König CH, Caro V, Guiso N, Pertussis PCR Consensus Group.** 2005.Nucleic acid amplification tests for diagnosis of *Bordetella* infections. J Clin Microbiol **43:**4925–4929.
4. **Register KB, Sanden GN.** 2006. Prevalence and sequence variants of IS*481* in *Bordetella bronchiseptica*: implications for IS*481*-based detection of *Bordetella pertussis*. J Clin Microbiol **44:**4577–4583. http://dx.doi.org/10.1128/JCM.01295-06.
5. **Tizolova A, Guiso N, Guillot S.** 2013. Insertion sequences shared by *Bordetella* species and implications for the biological diagnosis of pertussis syndrome. Eur J Clin Microbiol Infect Dis **32:**89–96. http://dx.doi.org/10.1007/s10096-012-1718-3.
6. **Caro V, Guiso N, Alberti C, Liguori S, Burucoa C, Couetdic G, Doucet-Populaire F, Ferroni A, Papin-Gibaud S, Grattard F, Réglier-Poupet H, Raymond J, Soler C, Bouchet S, Charreau S, Couzon B, Leymarie I, Tavares N, Choux M, Bingen E, Bonacorsi S.** 2009. Proficiency program for RT-PCR diagnosis of *Bordetella pertussis* infections in French hospital laboratories and at the French National Reference Center for Whooping Cough and other Bordetelloses. J Clin Microbiol **47:**3197–3203. http://dx.doi.org/10.1128/JCM.00817-09.
7. **Tubiana S, Belchior E, Guillot S, Guiso N, Lévy-Bruhl D, Renacoq participants.** 2015. Monitoring the impact of vaccination on pertussis in infants using an active hospital-based pediatric surveillance network: results from seventeen years' experience, 1996-2012, France. Pediatr Infect Dis J **34:**814–820. http://dx.doi.org/10.1097/INF.0000000000000739.
8. **Lanotte P, Plouzeau C, Burucoa C, Grélaud C, Guillot S, Guiso N, Garnier F.** 2011. Evaluation of four commercial RT-PCR assays for detection of *Bordetella* spp. in nasopharyngeal aspirates. J Clin Microbiol **49:**3943–3946. http://dx.doi.org/10.1128/JCM.00335-11.
9. **Dalby T, Fry NK, Krogfelt KA, Jensen JS, He Q, Pertussis EQA participants.** 2013. Evaluation of PCR methods for the diagnosis of pertussis by the European surveillance network for vaccine-preventable diseases (EUVAC.NET). Eur J Clin Microbiol Infect Dis **32:**1285–1289. http://dx.doi.org/10.1007/s10096.
10. **Njamkepo E, Bonacorsi S, Debruyne M, Gibaud SA, Guillot S, Guiso N.** 2011. Significant finding of *Bordetella holmesii* DNA in nasopharyngeal samples from French patients with suspected pertussis. J Clin Microbiol **49:**4347–4348. http://dx.doi.org/10.1128/JCM.01272-11.
11. **Rodgers L, Martin SW, Cohn A, Budd J, Marcon M, Terranella A, Mandal S, Salamon D, Leber A, Tondella ML, Tatti K, Spicer K, Emanuel A, Koch E, McGlone L, Pawloski L, Lemaile-Williams M, Tucker N, Iyer R, Clark TA, Diorio M.** 2013. Epidemiologic and laboratory features of a large outbreak of pertussis-like illnesses associated with cocirculating *Bordetella holmesii* and *Bordetella pertussis*–Ohio, 2010-2011. Clin Infect Dis **56:**322–331. http://dx.doi.org/10.1093/cid/cis888.
12. **Dinu S, Guillot S, Dragomirescu CC, Brun D, Lazăr S, Vancea G, Ionescu BM, Gherman MF, Bjerkestrand AF, Ungureanu V, Guiso N, Damian M.** 2014. Whooping cough in South-East Romania: a 1-year study. Diagn Microbiol Infect Dis **78:**302–306. http://dx.doi.org/10.1016/j.diagmicrobio.2013.09.017.
13. **Miranda C, Porte L, García P.** 2012. *Bordetella holmesii* in nasopharyngeal samples from Chilean patients with suspected *Bordetella pertussis* infection. J Clin Microbiol **50:**1505. http://dx.doi.org/10.1128/JCM.06747-11.
14. **Bottero D1, Griffith MM, Lara C, Flores D, Pianciola L, Gaillard ME, Mazzeo M, Zamboni MI, Spoleti MJ, Anchart E, Ruggeri D, Sorhouet C, Fiori S, Galas M, Tondella ML, Hozbor DF.** 2013. *Bordetella holmesii* in children suspected of pertussis in Argentina. Epidemiol Infect **141:**714–717. http://dx.doi.org/10.1017/S095026881200132X.