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Comparison of Serological and Real-Time PCR Assays To Diagnose *Bordetella pertussis* Infection in 2007▽

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Bacterial culture for diagnosing pertussis infection has high specificity but poor sensitivity and is slow. Highly sensitive real-time PCR assays and single-serum pertussis serology have been developed to overcome these limitations, but there are few data available on the relative sensitivities and specificities of such assays for pertussis diagnosis. Using data on 195 participants (≥7 years old) from an epidemiological study, we assessed the sensitivity, specificity, and performance (Youden index) for pertussis diagnosis of the pertussis toxin enzyme-linked immunosorbent assay (using single and paired serology) and of real-time PCR assays (using the IS*481* and *ptxA*-Pr targets). All available diagnostic information (clinical and laboratory) was pooled to serve as the gold standard. Single serology was the most efficient diagnostic test (Youden index, 0.57 to 0.58), with relatively high sensitivity (>64%) and high specificity (>90%), independent of the cutoff level. IS*481* PCR performance was superior to that of *ptxA*-Pr PCR, and it was the second-most-efficient tool (Youden index, 0.30). Performing both *ptxA*-Pr and IS*481* PCRs did not improve diagnostic performance. The greatest test efficiency (Youden index, 0.69 to 0.74) was achieved when single-serum serology was used in combination with IS*481* or *ptxA*-Pr PCR or paired serology. Combining single serology with one PCR or paired serology increased the sensitivity with an associated limited decrease in specificity. The most specific tests for diagnosis of pertussis were single serology and *ptxA*-Pr PCR, and the most sensitive diagnostic tool was the combination of IS*481* PCR with single serology.

Currently there is no satisfactory gold standard technique for laboratory confirmation of a pertussis infection. Although culture is highly specific, sensitivity is low and declines with the duration of illness, and the method may take up to 7 days to provide a result. Using culture alone to diagnose pertussis is likely to lead to underreporting of pertussis cases (8). Serological assays, using one or two serum samples, have been developed to improve the sensitivity of the pertussis diagnosis, but they have a lower specificity than culture (3, 9, 20, 25). PCR techniques were developed to overcome these limitations, but despite a consensus meeting in 2005 (24), these techniques still require further standardization and optimization. To date there are few reports (8) in which the sensitivities and specificities of these different techniques are compared using a population exposed to pertussis.

The objective of this study was to compare the sensitivities and specificities of the current most widely used techniques to diagnose pertussis in exposed populations, namely, two PCR methods (IS*481* and *ptxA*-Pr targets) and single and paired serology for detection of antipertussis toxin (PT) antibodies in the serum of the patients using enzyme-linked immunosorbent assay (ELISA) with two different cutoffs (two- or fourfold change for paired serology and ≥100 or ≥125 ELISA units [EU]/ml for single serology).

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MATERIALS AND METHODS

Patients and specimens. We used data obtained in the course of a recent prospective multicenter epidemiologic study (27). This study included 404 household contacts of 94 young infants (≤6 months of age) with laboratory-confirmed pertussis. All contacts were interviewed face-to-face using a standard questionnaire to obtain relevant demographic and clinical data, particularly the presence and duration of symptoms of cold or cough during the month prior to their inclusion. They also provided nasopharyngeal aspirates or, in a few centers, swab samples for culture and/or PCR detection of *Bordetella pertussis* and an acute blood sample for detection of anti-PT immunoglobulin G (IgG). One month later, data on the presence of cough and cold-like symptoms and a convalescent-phase blood sample were collected from participating contacts. All nasopharyngeal aspirates, swabs, and sera were sent to our laboratory for analysis with real-time PCR (using the IS*481* target or the *ptxA*-Pr target) and measurement of anti-PT IgG by ELISA.

Culture and analysis of isolates. Culture was performed in only a few participating centers, resulting in five isolates from France, three from Germany, and five from the United States. Isolates were analyzed by pulsed-field gel electrophoresis (PFGE) and genotyping (7) and expression of bacterial toxins and adhesins analyzed using specific poly- and monoclonal antibodies (16, 22).

PCR. (i) Detection of *B. pertussis* by real-time PCR targeting the pertussis toxin promoter (*ptxA*-Pr) using hybridization probes. (a) Oligonucleotide primers and hybridization probes. Oligonucleotide primers were adapted from the work of Grimprel et al. (13) and manufactured by TibMolBiol (Berlin, Germany). A pair of fluorescence-labeled hybridization probes (TibMolBiol), PT2-FLU (5′-CCT CGA TTC TTC CGT ACA TCC CGC TAC T-3′ fluorescein) and PT2-LCR640 (5′ LCred640-AAT CCA ACA CGG CAT GAA CGC TCC TTC-3′ phosphate group) were designed and used for the real-time detection of the *ptxA*-Pr-specific PCR product. A third probe, ICPT-LCR (5′ LCred705-TGA CTG ACA TCA GGC TTG CGC ATT GCT A-3′ phosphate group), was

designed for the detection of the specific internal-control DNA constructed for this real-time PCR assay.

(b) Internal control. PCR inhibition was assessed by the addition of 4 fg of our specific internal control DNA (ICD-PT) to each sample tested, which was detected in duplex format. The strategy used to construct the internal control DNA (ICD) is the overlap extension technique (15), which allows construction of an ICD with the same primer binding sequences as the target DNA and containing a nontarget nucleic acid sequence (chimeric DNA). The constructed ICD is shorter (152 bp) than a natural PCR product and is inserted in the pCR4Blunt-TOPO plasmid (Invitrogen, California) to provide an unlimited amount of ICD. Furthermore, a specific probe (labeled with LCred705) could be designed as an ICD-PT containing chimeric DNA. The use of the same primers is an advantage, since multiple sets of primers might interfere with the amplification of one or both of the target genes.

The inclusion of 4 fg ICD-PT in the PCR mixture did not affect the sensitivity of the PCR for the detection of a *ptxA*-Pr-specific PCR product and allowed correct detection of PCR failure.

(c) *ptxA*-Pr PCR amplification. PCR amplification was performed using a real-time PCR system (LightCycler; Roche). The 20-μl reaction mixture volume, in a glass capillary tube, contained 4 μl FastStart reaction mix hybridization probes (a component of the FastStart DNA Master^{PLUS} hybridization probe kit; Roche Diagnostics), 0.4 μl dimethyl sulfoxide, 1 U uracil-DNA glycosylase, 0.5 μM (each) primers PT-1 and PT-2, 0.2 μM (each) probes PT2-LCR640 and ICPT-LCR, and 0.4 μM probe PT2-FLU.

Reaction conditions were 10 min at 95°C, followed by 50 cycles of 5 s at 95°C, 5 s at 66°C, and 8 s at 72°C. The fluorescence increase was measured during the annealing step at 66°C. A readout of LC-Red 640 values (*B. pertussis*-specific product) was performed using channel F2/back-F1 software, and a readout of LC-Red705 values (internal control DNA-specific product) was performed using channel F3/back-F2 software.

(d) Analytical sensitivity and specificity. A detection limit between 50 fg (equivalent to 10 CFU) and 5 fg (equivalent to 1 CFU) of *B. pertussis* template DNA was observed. No detection of the seven other *Bordetella* species, nor of non-*Bordetella* respiratory organisms, i.e., the *Alcaligenaceae* (*Achromobacter* spp. and *Alcaligenes* spp.) or *Moraxellaceae* (*Branhamella* spp. and *Moraxella* spp.) families or *Pasteurella* spp. or *Neisseria* spp., was observed, confirming our previous data (13).

(ii) Detection of *B. pertussis* by real-time PCR targeting IS*481* using hybridization probes. The real-time PCR using IS*481* as the target was performed according to the recommendations of the PCR consensus meeting (24).

Serology. Serum samples were analyzed using ELISA to quantify IgG anti-PT antibody, as previously described (25), using purified PT from Sanofi Pasteur. The assay cutoff was set at 20 EU/ml. Positive paired serology was defined with different cutoffs: a two- or fourfold change (25) in anti-PT IgG titers between acute- and convalescent-phase serum samples. Positive single serology was defined as a single anti-PT IgG antibody titer of ≥100 or 125 EU/ml (3). Anti-PT serology results were not used to confirm pertussis in immunized children 3 months to <2 years of age and 4 to 7 years of age, since they may be influenced by recent pertussis vaccination.

Pertussis case definition. Contacts were classified as the following: (i) symptomatic, laboratory-confirmed pertussis cases, (ii) symptomatic, epidemiologically linked pertussis cases (18a), (iii) contacts with asymptomatic or subclinical pertussis infection, or (iv) contacts without evidence of recent pertussis infection. Epidemiologically linked cases were defined as persons in contact with the infant index case in the month preceding symptom onset in the index case which had an acute cough illness lasting ≥2 weeks but no laboratory confirmation of pertussis. Laboratory-confirmed "asymptomatic" or subclinical cases of pertussis met the same criteria as laboratory-confirmed symptomatic cases but did not report any cough or cold symptoms.

The case definition for symptomatic laboratory-confirmed cases was based on a modification of the algorithm developed by Wendelboe et al. (27). In the current study, a case was classified as a symptomatic laboratory-confirmed case when symptomatic and positive by culture, IS*481* PCR, *ptxA*-Pr PCR, single serum (≥125 EU/ml), or paired serology (≥4-fold change in ELISA titer).

Laboratory confirmation was defined as at least one positive culture, PCR (IS*481* or *ptxA*-Pr), or positive single or paired serology.

Statistical analysis. To avoid bias in interpretation associated with the difficulties in interpreting serology with immunized children between 3 months and 7 years of age (21 participants) and with nonimmunized infants less than 3 months old (2 participants), the main analysis was performed with the 195 participants older than 7 years of age, with complete laboratory diagnostic information both at inclusion (*ptxA*-Pr PCR, IS*481* PCR, and serology) and 1 month later (second serology). We first calculated the sensitivity and the specificity of each of the individual laboratory techniques including *ptxA*-Pr PCR, IS*481* PCR, single serology with IgG antibody titers of ≥125 EU/ml, and paired serology with a fourfold change in IgG titers. For the calculation of the sensitivity of the diagnostic test *i*, true-positive cases were defined as symptomatic laboratory-confirmed cases with positive diagnostic test *i* and false-negative cases were defined as symptomatic laboratory-confirmed cases with a negative result on diagnostic test *i*. For the calculation of the specificity of a diagnostic test, true-negative cases were defined as epidemiologically linked pertussis cases, contacts with asymptomatic or subclinical pertussis infection, or contacts without evidence of recent pertussis infection with a negative diagnostic test *i* and false-positive cases as epidemiologically linked pertussis cases, contacts with asymptomatic or subclinical pertussis infection, or contacts without evidence of recent pertussis infection with a positive diagnostic test *i*.

We then determined the sensitivities and specificities of the combinations of different tests, including single and paired serology and PCR assays (*ptxA*-Pr or IS*481*) with single serology to estimate the ability to diagnosis pertussis using data collected at first presentation. Sensitivities and specificities were reported with 95% confidence intervals and compared using the Student *t* test. Key demographic and clinical characteristics of participants with and without missing laboratory data were compared using the chi-squared test. Laboratory diagnostics were ranked according to the Youden index (28), a summary measure of the reciprocal-operating-characteristics curve, to estimate the effectiveness of the diagnostic test. The Youden index was calculated as follows: (sensitivity + specificity) − 1. A perfect diagnostic test, i.e., a test which allows complete separation of the diseased and healthy populations, has a Youden index of 1 (12).

Finally, we performed one sensitivity analysis, using a case definition with the other reported cutoff for single serology (IgG antibody titers of ≥100 EU/ml) and paired serology (twofold change in ELISA titers) with the same case and noncase definitions.

TABLE 1. Status of completeness for pertussis laboratory diagnosis variables: *ptxA*-Pr PCR, IS*481* PCR, and serology

Description	No. of subjects
Complete data at inclusion visit	256
Data at second visit	218
Missing data at second visit	38
Missing data at inclusion visit	148
On serology	17
On PCR	97
On both PCR and serology	34
Total	404

RESULTS

Description of the analyzed population. Among the 404 contacts included in the epidemiological study, 353 participants provided a serological sample at the inclusion visit. Within this group, 14 participants, of ages from 3 months to 2 years or 4 to 7 years, were excluded from the analysis. *ptxA*-Pr PCR and IS*481* PCR were performed on samples from 289 and 288 participants, respectively. Complete laboratory data sets were available for 256 participants at the inclusion visit and 218 participants for both visits (Table 1). Compared with those with complete diagnostic data, participants with missing data were more likely to be male (52% versus 39%; $P = 0.02$), <18 years old (52.0% versus 30.7%; $P < 0.0001$), and symptomatic for pertussis (72.3% versus 60.1%; $P = 0.03$) (Table 2). Among the 218 contacts analyzed (of whom 195 were ≥7 years old, 21 were ≥3 months old and <7 years old, and 2 were <3 months old), 95 (of whom 82 were ≥7 years old) had a laboratory-confirmed symptomatic pertussis infection, 14 (of whom 10 were ≥7 years old) had a symptomatic epidemiologically linked pertussis infection, 33 (of whom 32 were ≥7 years old)

TABLE 2. Description of participants according to existing missing data on laboratory diagnosis of pertussis infection

Characteristic	Value for group		*P* value
	Missing-data group	Laboratory complete-data group	
Total participants	148	218	
Clinical symptoms present, % (no.)	72.3 (107)	60.1 (131)	0.03
Sex distribution, % (no.)			0.02
Men	52.0 (77)	39.0 (85)	
Women	48.0 (71)	61.0 (133)	
Age (median), yrs	15.5	27.0	
Age (2 classes), % (no.)			0.0001
Adults, ≥18 yrs	48.0 (71)	69.3 (151)	
Children, <18 yrs	52.0 (77)	30.7 (67)	
Age (4 classes), % (no.)			0.00001
<10 yrs	41.2 (61)	14.2 (31)	
≥10 yrs–<18 yrs	10.8 (16)	16.5 (36)	
≥18 yrs–<50 yrs	42.6 (63)	60.1 (131)	
≥50 yrs	5.4 (8)	9.2 (20)	
Continent, % (no.)			0.89
North America	67.6 (100)	68.8 (150)	
Europe	32.4 (48)	31.2 (68)	

had an asymptomatic or subclinical pertussis infection, and 76 (of whom 71 were ≥7 years old) were totally free of pertussis infection.

Analysis of isolates collected during the study. Only 13 isolates were collected during the study. They were collected nine times for index cases and four times for mothers of index cases. These adults had all PCR- and serology-positive diagnoses. All isolates express the characterized toxins and adhesins. Genotyping of the PT S1 subunit showed that they all harbored similar *ptxA1* and *prn2* genes except for one U.S. isolate harboring a *prn3* gene. We previously separated *B. pertussis* isolates into five major PFGE groups (6, 7, 25). All 13 isolates were included in the same PFGE group, group IV. All U.S. and French isolates were characterized as PFGE subgroup IV beta with one exception, which was PFGE subgroup IV gamma, and the three German ones were either PFGE subgroup alpha or beta.

Comparative sensitivities and specificities of real-time PCR and serological assays for diagnosis of pertussis infection. (i) Main analysis. The number of positive tests by type of contact is presented in Table 3. The sensitivity and specificity of each diagnostic assay are presented for the 195 participants with complete data at both visits (Table 4).

Among PCR assays, the sensitivity of the *ptxA*-Pr PCR was significantly lower than the sensitivity of the IS*481* PCR (15.9% versus 45.1%; $P < 10^{-9}$), whereas the specificity of the *ptxA*-Pr PCR was significantly higher than that for IS*481* PCR (97.3% versus 85.0%; $P < 10^{-5}$).

Single serology was significantly more sensitive (64.6% versus 15.9%; $P < 10^{-9}$)) than paired serology and had comparable specificity (92.0% versus 89.4%; $P < 0.19$. Single serology was also more sensitive than the IS*481* PCR (64.6% versus 45.1%; $P < 10^{-4}$) and the *ptxA*-Pr PCR (64.6% versus 15.9%; $P < 10^{-9}$).

Single serology was more specific than IS*481* PCR (92.0% versus 85.0%; $P < 0.02$) but less specific than the *ptxA*-Pr PCR (92.0% versus 97.3%; $P < 0.01$).

TABLE 3. Distribution of positive and negative cases with laboratory-confirmed pertussis for participants ≥7 years old[a]

Method of diagnosis (cutoff)	No. of cases	No. of noncases
Positive pertussis test		
ptxA-Pr PCR	13	3
IS*481* PCR	37	17
Single serology (≥125 EU/ml)	53	9
Paired serology (×4[b])	13	12
Positive pertussis test using combination of single serology (≥125 EU/ml) with:		
Paired serology (×4)	66	12
PtxA-Pr PCR	62	12
IS*481* PCR	73	23

[a] Subjects were contacts of infant pertussis cases with complete laboratory data (n = 195). Complete data include symptoms, serology, and PCR (IS*481* and *ptx*-Pr) at enrollment and symptoms and serology at follow-up visit 1 month later.
[b] ×4, fourfold change in titer.
[c] Case definition: laboratory-confirmed symptomatic contact. There were 82 cases and 113 noncases.

Among all individual diagnostic assays, single serology was the most efficient assay, with Youden indices of 0.57, compared to <0.31 for all other assays.

As summarized by the Youden indices (Table 4), the most efficient laboratory diagnoses for symptomatic laboratory-confirmed pertussis cases were the combination of single serology with paired serology or one PCR test. The most sensitive test was the combination of single serology with IS*481* PCR, and the most specific was the combination of single serology with *ptxA*-Pr PCR.

(ii) Sensitivity analyses. Using more-sensitive cutoffs for serology (≥100 and a twofold change), there were 84 cases and 111 noncases. Lowering the single-serum cutoff to ≥100 EU/ml or the cutoff for paired serology to a ≥2-fold change in titer slightly improved efficiency of individual tests due to a modest increase in sensitivity and a small decrease in specificity; nevertheless, the results for the estimated sensitivity and specificity of the laboratory tests were comparable to those reported in the main analysis (Table 5). The most specific diagnostic tests were the combination of single serology with *ptxA*-Pr PCR or IS*481* PCR, and the most sensitive were the combination of single serology with paired serology or with the IS*481* PCR.

DISCUSSION

This study was performed in order to compare different pertussis biological diagnostic techniques in current routine use. The source data were obtained from a multicenter household study on pertussis transmission (27) in which five diagnostic measures were used: culture, two PCR assays with different DNA targets, and single and paired PT ELISAs with two cutoffs.

Culture was not performed in all centers because of the difficulty in isolating *B. pertussis*, the lack of sensitivity, and the long delay necessary to obtain the result (8). However, it is important to note that pursuing culture is essential in order to

TABLE 4. Comparative sensitivities, specificities, and Youden index for diagnosis of symptomatic laboratory-confirmed pertussis[a]

Method of diagnosis (cutoff)	% Sensitivity (95% CI[c])	% Specificity (95% CI)	Youden index
ptxA-Pr PCR	15.9 (10.8–21.0)	97.3 (95.1–99.5)	0.13
IS*481* PCR	45.1 (38.1–52.1)	85.0 (80.0–90.0)	0.30
Single serology (≥125 EU/ml)	64.6 (57.9–71.3)	92.0 (88.2–95.8)	0.57
Paired serology (×4[b])	15.9 (10.8–21.0)	89.4 (85.1–93.7)	0.05
Combination of single serology (≥125 EU/ml) with:			
Paired serology, (×4)	80.4 (74.8–86.0)	89.4 (85.1–93.7)	0.70
ptxA-Pr PCR	75.6 (69.6–81.6)	98.2 (96.3–100)	0.74
IS*481* PCR	89.0 (84.6–93.4)	79.6 (73.9–85.3)	0.69

[a] Subjects are contacts of infant pertussis cases for whom there is complete laboratory data ($n = 195$). Complete data include symptoms, serology, and PCR (IS*481* and *ptx*-Pr) at enrollment and symptoms and serology at follow-up visit 1 month later.
[b] ×4, fourfold change in titer.
[c] CI, confidence interval.

analyze the spatio-temporal evolution of *Bordetella pertussis* according to the immunity of the human population. We observed that the collected isolates were very similar to each other (all PFGE group IV), confirming our previous results obtained in Argentina (4), Europe (7), Japan (14), Russia (17), and France (6, 26). We demonstrated that the PFGE group IV subgroup gamma, first observed in Finland and more recently in France (5, 7), is now also circulating in the United States.

Because of the long delay necessary to isolate the bacteria, many real-time PCR assays have been developed in recent years for routine diagnoses of pertussis. After a consensus meeting in 2005, recommendations were published (24). It was underlined that real-time *ptxA*-Pr PCR was less sensitive but more specific than IS*481* PCR (24), since IS*481* PCR can detect *Bordetella holmesii* species and some *Bordetella bronchiseptica* and *B. pertussis* spp. This may not impact substantially on test performance, since the incidence of *B. holmesii* respiratory infections seems to be very low, as is that of *B. bronchiseptica*, which infects mostly immunosuppressed individuals (2, 11). In the present study, we confirmed that *ptxA*-Pr real-time PCR is less sensitive than IS*481* PCR and demonstrated that performing both *ptxA*-Pr and IS*481* PCR does not improve diagnostic performance. Furthermore, we did not observe any *ptxA*-Pr PCR-positive case which was negative by IS*481* PCR. This observation is in contrast with that of Qin et al. (23).

Qin et al. speculate that the underperformance of IS*481* compared to that of the multicopy IS may have been a result of nonrandom genome degradation of IS*481*, which is considered a nonessential genomic element, during the course of host-pathogen interaction at a later disease stage. The difference between the two studies may also be explained by a difference in the study population, with the Qin study including mainly young children (median age, 2.5 years) suspected of having pertussis infection, whereas our study included contacts of infant index cases (median age, 27 years) investigated at a later stage of disease. However, even among those contacts that were assessed when symptomatic for a short period of time, we did not observe any *ptxA*-Pr PCR-positive case which was negative by IS*481* PCR. Our data confirm that IS*481* real-time PCR is a useful diagnostic tool.

ELISA determinations of anti-PT antibody titers are a validated and very sensitive diagnostic method which can be used with single serum or paired (acute- and convalescent-phase) serum samples (8). A fourfold change in the titers of anti-PT antibodies was believed to be the most sensitive and specific biological diagnosis (25). However, the paired serum sample assay is impractical for routine diagnosis. For this reason, single-sample serology has been developed and IgG cutoff values have been determined for a number of laboratories (3, 8, 9), and this method is now used for routine diagnosis. In the present study, we compared single and paired serology and two different cutoffs. Twofold changes were more sensitive but less specific than fourfold changes. Single serology, independently of the cutoff used, was more sensitive and more specific than paired serology, and the agreement was poor between single and paired serology results. We demonstrated that the perfor-

TABLE 5. Comparative sensitivities, specificities, and Youden index for diagnosis of symptomatic laboratory-confirmed pertussis[a]

Method of diagnosis (cutoff)	% Sensitivity (95% CI[c])	% Specificity (95% CI)	Youden index
ptxA-Pr PCR	15.5 (10.4–20.6)	97.3 (95.0–99.6)	0.13
IS*481* PCR	44.0 (37.0–51.0)	84.7 (79.6–89.8)	0.29
Single serology (≥100)	67.9 (61.3–74.5)	90.1 (85.9–94.3)	0.58
Paired serology (×2[b])	23.8 (17.8–29.8)	83.8 (78.6–89.0)	0.08
Combination of single serology (≥100 EU/ml) with:			
Paired serology (×2)	85.7 (80.8–90.6)	75.7 (69.7–81.7)	0.61
PtxA Pr PCR	77.4 (71.5–83.3)	87.4 (82.7–92.1)	0.65
IS*481* PCR	90.5 (86.4–94.6)	77.5 (71.6–83.4)	0.68

[a] Subjects are contacts of infant pertussis cases for whom there is complete laboratory data ($n = 195$). Complete data include symptoms, serology, and PCR analysis (IS*481* and *ptx*-Pr) at enrollment and symptoms and serology at follow-up visit 1 month later.
[b] ×2, twofold change in titer.
[c] CI, confidence interval.

mance of the combination of single serology with paired serology did not improve the performance of single serology and confirmed the usefulness of single serology for routine diagnosis of pertussis.

This study is subject to some limitations. Culture has traditionally served as an imperfect gold standard, but its poor sensitivity limits the ability to assess the performance of other diagnostic tests. Hence, an algorithm was developed which incorporated diagnostic information from all available sources (i.e., PCR, serum, and symptoms). While the use of an algorithm as a pooled gold standard is superior to treating a single imperfect diagnostic test as an absolute gold standard, using a pooled gold standard potentially results in overestimating the sensitivities and specificities of the individual tests included in the pooled gold standard (21). To compare the values of the different diagnostic tests, we presented the sensitivity, specificity, and Youden index for each of the tests assessed. The Youden index is an attractive single value for the effectiveness of a diagnostic marker in distinguishing between diseased and nondiseased populations. A limitation of the Youden index stems from the fact that it gives equal weight to the sensitivity and specificity. Depending on the setting in which the test is used, a health care provider or researcher may want to give greater weight to either the sensitivity or specificity of a particular diagnostic test.

All the participants included in these analyses were close contacts of confirmed cases of pertussis infection who had a higher probability of being exposed to *B. pertussis* than the general population and therefore had a higher probability of having a positive laboratory test.

In addition, this study design could have produced a relatively large number of asymptomatic contacts with positive laboratory tests, thus biasing the estimate of the sensitivity and specificity of these laboratory tests compared to those for the general population. Further research is needed to determine if the same levels of sensitivity and specificity and the same ranking of test efficacy are obtained with the general population.

In conclusion, IS*481* PCR and single-serum serology are currently the most efficient diagnostic tools available for pertussis diagnosis for children of ages ≥7 years, adolescents, and adults in contact with a pertussis case. The combination of both assays provides the most sensitive diagnostic tool for pertussis at the time of presentation to a health care facility. Culture will remain valuable since it allows for the analysis of the spatiotemporal evolution of the bacterium.

The efficiency of single serology may require future reevaluation, however, because of changes in pertussis vaccines (replacement of whole-cell vaccines with acellular ones) and new vaccine recommendations which promote booster vaccinations for adolescents and adults (1, 18). Acellular vaccines contain fewer antigens than whole-cell vaccines and induce different and larger humoral immune responses than whole-cell vaccines (10). The use of single serum may thus decrease with more-widespread implementation of booster vaccinations, since serological diagnoses can be performed only after a waiting period of 2 to 3 years following a booster immunization with a pertussis acellular vaccine (19).

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