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Aerosolized Vaccine as an Unexpected Source of False-Positive *Bordetella pertussis* PCR Results

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When 13 of 13 nasal wash specimens from a single pediatrician's office tested positive for low quantities of *Bordetella pertussis* DNA, we suspected prelaboratory contamination. Investigation revealed that Pentacel and Adacel vaccines contain high copy numbers of *B. pertussis* DNA, which can be aerosolized, causing false-positive *B. pertussis* PCR results.

Pertussis, caused by *Bordetella pertussis*, is a vaccine-preventable, reportable disease in the United States. Its incidence is increasing (1). Numbers of cases in Michigan rose from 315 in 2008 to 1,564 in 2010 (9). Because culture and confirmation for *B. pertussis* may take up to 2 weeks, and is no more than 60% sensitive, timely diagnosis has come to rely heavily on PCR (3). Many clinical laboratories successfully perform diagnostic PCR every day. However, PCR for *B. pertussis* has been subject to significant issues with contamination (1, 2, 3, 4, 8), including one hospital-based pseudo-outbreak that resulted in 1,700 health care provider (HCP) visits to employee health services and postexposure prophylaxis for 1,300 of their contacts (1). Part of the explanation is the use of single-target PCR assays targeting IS*481*, an insertion sequence found in multiple copies in *B. pertussis*. IS*481* is also found in lower copy numbers in *Bordetella holmslei* and *Bordetella bronchiseptica*, both of which can cause pertussis-like illnesses (1, 3, 10, 11). Use of this sequence continues because the number of infectious organisms after a few weeks of infection is very low and the use of a single copy gene would reduce the sensitivity of the test (3, 8). Amplicon contamination in PCR laboratories causes false-positive results (8, 12), but these have been reduced since the advent of real-time PCR (rtPCR), which does not require the opening of tubes postamplification (6). It has recently been shown that some, but not all, *B. pertussis* vaccines contain genomic DNA in addition to bacterial antigens (7, 13).

The microbiology laboratory at Detroit Medical Center University Laboratories, which performs approximately 355,000 molecular tests/year, developed and validated a qualitative, multiplex, rtPCR assay (available as analyte-specific reagents from EraGen Biosciences, Madison, WI). The assay detects both *B. pertussis* (target, IS*481*) and *Bordetella parapertussis* (target, IS*1001*). After 50 cycles of amplification are carried out with the LightCycler v2.0 (Roche Diagnostics, Indianapolis, IN), amplicon melting curves are used to distinguish the targets. Qualitative rtPCR assays allow approximate quantification of the target DNA present. The lower the cycle threshold (CT) at which amplification product is first detectable, the higher the concentration of target DNA.

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Original pediatrician's office. In December, 2010, 13 of 13 nasal wash specimens, received from a single physician's office over a 2-day period, were positive for *B. pertussis*, with CT values of 38 to 40.9 cycles. Based on our studies of the assay's sensitivity and linearity, these CTs indicate titers of ≤100 genomes/ml. At that time, routine laboratory wipe tests (5), reagent blanks, and specimens from other physician offices were negative, suggesting that laboratory contamination was not the cause. Investigation by the Michigan Department of Community Health revealed that most of the children did not exhibit symptoms compatible with pertussis. Some children, tested on the second day, lacked respiratory symptoms but were tested because they were contacts of children with prior positive *B. pertussis* PCR results. The physician's nurse reported that the 13 nasal wash specimens were collected in the same rooms where vaccination preparation and injection routinely took place. We do not know whether any of these children were vaccinated during the visits when their diagnostic samples were collected or whether other patients had been vaccinated earlier that day in the same exam rooms.

A laboratory representative went to the pediatrician's office and returned with materials used for collection of nasal wash specimens and an empty vial of the diphtheria and tetanus toxoids, acellular pertussis, inactivated poliovirus (DTaP-IPV) component of the Pentacel (Sanofi Pasteur [SP], Swiftwater, PA) pediatric vaccine. Prior to vaccine administration, the entire liquid contents of this vial are used to reconstitute the lyophilized contents of the second vial, which contains protein-conjugated capsular antigen from *Haemophilus influenzae*, type b.

To detect *B. pertussis* DNA, sterile, deionized, PCR-grade water (500 μl) was used to rinse the empty vaccine vial. To sample the dry components of the collection kits, skin, and other dry surfaces tested in this study, 500 μl of PCR-grade water was placed in a sterile tube. A sterile swab was moistened with this water, used to wipe the test surface, swirled vigorously in the tube, and discarded (5). Nucleic acids were extracted from 200 μl of each specimen with an EasyMag system (bioMérieux, Durham, NC) by following the manufacturer's instructions. This concentrates specimens to 50 μl, of which 5 μl is used in each *B. pertussis* PCR tube.

All sealed sample collection materials from the physician's office were negative when tested by our *B. pertussis* PCR assay. The

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TABLE 1 *Bordetella pertussis* PCR results of original patient specimens and other samples

Source and specimen	CT ± SD[a]	Interpretation
Original pediatrician's office		
Original patients ($n = 13$) (mean CT)	39.33 ± 0.98	Positive[b]
Nasal wash solution	—[c]	Negative
Bulb	—	Negative
Pentacel DTap-IPV washing[d]	29.13	Positive
Unopened vaccine bottles[d]		
Adacel (1:100)	17.7 ± 0.2	Positive
Pentacel DTap-IPV (1:100)	15.1 ± 0.13	Positive
Infanrix (undiluted)	—	Negative
Pediatric vaccine clinic[e]		
HCP A (nares; before work)	—	Negative
HCP A (nares; after work)[f,g]	40.3	Positive
HCP A (hand; before work)	—	Negative
HCP A (hand; after work)[f,g]	36.1	Positive
HCP B (nares; before work)	—	Negative
HCP B (nares; after work)[f,g]	39.8	Positive
HCP B (hand; before work)	—	Negative
HCP B (hand; after work)[f,g]	37.0	Positive
Computer keyboard (wipe)[f,g,h]	36.8	Positive
Preparation table (wipe)[f,g,h]	32.5	Positive
Examination table (wipe)[f,g,h]	34.0	Positive
Fridge handle (wipe)[f,g,h]	34.6	Positive
Wall above prepn table (wipe)[f,g,h]	32.0	Positive

[a] CT, cycle threshold (see text). If a standard deviation (SD) is given, it is based on three determinations unless otherwise specified.
[b] Positive, positive for *Bordetella pertussis*.
[c] —, not detected.
[d] Prepared as described in the text. Adacel and Pentacel were from Sanofi Pasteur, and Infanrix was from GlaxoSmithKline.
[e] A and B, identification codes for two HCPs whose nares or hands were tested before or after the workday, as indicated; wipe, wipe tests of the indicated locations.
[f] Specificity for *B. pertussis* was confirmed by PCR for the promoter region of the toxin A gene.
[g] Wipe test, see text.
[h] Selected from 20 environmental wipe test samples, all of which were positive.

wash from the Pentacel DTaP-IPV vial was positive, with a CT of 29.13 (Table 1, top).

Unopened vaccines. Unopened vials of three vaccines were obtained: the DTaP-IPV component of Pentacel, Adacel (also SP; lacks IPV and *H. influenzae* antigens; for individuals >11 years old), and Infanrix (GlaxoSmithKline, Research Triangle Park, NC). They were tested undiluted and after a 1:100 dilution (Table 1, middle). Undiluted Infanrix was negative for *B. pertussis* DNA. Diluted Pentacel and Adacel were positive, with CTs of 15.1 ± 0.13 and 17.7 ± 0.2 cycles, respectively. Our positive *B. pertussis* control (approximately 10,000 CFU/ml) had a CT of 34.2 cycles, about 19 cycles later. Factoring in the 100-fold dilution of the vaccine sample, and assuming perfect efficiency at each PCR cycle and the same number of targets/organism as our control *B. pertussis*, the Pentacel vaccine contains the equivalent of approximately 5×10^{11} CFU/ml. This is only an approximation, because the number of IS*481* targets in different *B. pertussis* strains is not known, nor is there any evidence to indicate the extent of fragmentation of the *B. pertussis* DNA in the vaccine.

Pediatric clinic. Subsequent quality control investigations were carried out with the cooperation of the staff at a Detroit Medical Center pediatric vaccine clinic that utilizes both Adacel and Pentacel. Before starting work after several days off, and again at the end of the workday, two HCPs swabbed their hands and anterior nares as described above. The initial four specimens were negative, while those collected at the end of the work day were positive (Table 1, bottom). Twenty wipe-test specimens, collected from areas within the clinic at the end of the day (five are shown in Table 1, bottom) were positive for *B. pertussis* DNA by PCR. Many of the objects swabbed were routinely touched by the hands of the health care providers, but finding *B. pertussis* genomic DNA on an inaccessible wall above the preparation area suggested aerosol contamination.

Confirmation. Because PCR for IS*481* is not specific for *B. pertussis*, we sent a Pentacel vaccine sample, several environmental samples (including samples from the wall), and samples from the hands and nares of the HCPs to a reference laboratory that uses a specific PCR test targeting the *B. pertussis* toxin A promoter region. All were reported as positive.

We confirm that Adacel and Pentacel vaccines contain amplifiable *B. pertussis* DNA, while Infanrix does not. Finding amplifiable *B. pertussis* DNA on the hands and in the anterior nares of two healthy HCPs after they prepared and administered the vaccine to multiple, apparently healthy children, lead us to the hypothesis that aerosol transmission could have been involved in our earlier patients' falsely positive nasal wash specimens. Swabs of the anterior nares are not recommended for diagnosis of *B. pertussis* infection, but we did not suspect that these healthy HCPs were infected. Finding *B. pertussis* genetic material in their nares provides evidence of airborne transmission of the material. Likewise, the finding of *B. pertussis* DNA on an inaccessible wall above the vaccine preparation area further supports this conclusion. The elimination of air bubbles from the syringe prior to injection generates aerosols, making contamination of the room air inevitable.

While this study was in preparation, the CDC published "Best Practice Recommendations for Health Care Professionals" (3). They recommended that specimen collection for *B. pertussis* be carried out in an area separate from that used for vaccine preparation and administration, that gloves be worn during specimen collection or vaccine administration and discarded immediately, and that clinic surfaces be cleaned using a 10% bleach solution. We support these recommendations, especially the collection of *B. pertussis* samples in a room that is never used for vaccine preparation and administration. This would probably have prevented the contamination of 13 of the 13 nasal wash specimens, which had initially led us to suspect aerosol contamination.

Based on the assumption that specimen contamination comes from the hands of HCPs, the CDC made two additional recommendations (3). First, they recommended that, to avoid transfer of DNA in the vaccine from the hands or gloves of HCPs into the specimen, either the nasopharyngeal swab for *B. pertussis* testing should be sent to the lab dry or the person collecting the sample should touch the handle only above the point at which they will break it off. We prefer breaking off the swab handles after specimen collection, because sending swabs with contaminated handles to the PCR laboratory may contaminate the laboratory. Second, the CDC noted that nasal washes are preferable, because they result in the best specimen and because they are less apt to be contaminated by DNA present on the hands of HCPs. The last two recommendations may not necessarily prevent aerosolized contamination. But the CDC recommendation for administering and

collecting specimens in separate rooms would likely prevent introduction of aerosolized vaccine into a nasal wash specimen.

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