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Contamination of Qiagen DNA Extraction Kits with *Legionella* DNA

van der Zee and Crielaard recently reported the contamination of Qiagen DNA extraction columns with *Legionella* species (6). We have independently made this discovery and have attempted to further characterize the contaminating DNA.

We used the Qiagen columns (QIAamp DNA minikit; Qiagen, Hilden, Germany) for the extraction of DNA from a variety of clinical samples to be tested for *Legionella* DNA by PCR. When PCR assays targeting the *Legionella* 16S rRNA gene (1) or 5S rRNA gene (2) were used, positive signals were frequently recorded for the negative controls. The 5S rRNA amplicon contained a single *Taq*I restriction site, a characteristic of *Legionella* spp. that we have used to confirm the specificity of the PCR assay (2). Investigation of all potential sources of contamination indicated that the columns were the source and that contamination varied with different batches of columns. The 16S rRNA amplified product was sequenced by using primers for the *Legionella* 16S rRNA gene (1) and the Sanger dideoxy chain terminator method (5), and the sequence obtained was compared with those submitted to the GenBank database by using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The closest matches for this sequence (with 96% identity) were *Legionella*-like amoebal pathogen (LLAP) strains 2, 3, 6, 7, 9, and 14, *Legionella lytica* strain L2, and *Legionella birminghamensis*. The alignment of the sequences from the contaminating product and these organisms can be seen in Table 1.

A PCR product was also obtained from a negative Qiagen extraction control, sequenced in the 23S-5S rRNA region (4), and submitted for identification in the same manner. Of the 312 bp of sequence submitted, only 246 bp shared any similarity with any reference species that had been submitted to the National Center for Biotechnology Information BLAST database. It was noted that no LLAP sequences for the 23S-5S rRNA region had been submitted; this may explain the poor degree of identification obtained. Alternatively, the product amplified in this region may not have been from the same source (organism) as that obtained in the 16S rRNA region. Limiting the sequence identification to the 5S rRNA region gave a 96% match with *Legionella steigerwaltii* (Z30463).

Sequencing using eubacterial primers (3) was also attempted, but this PCR was not adequately sensitive to detect a product in the water extract.

We agree with van der Zee and Crielaard (6) that the Qiagen columns are likely contaminated during production through the process of flushing with water containing *Legionella* spp. or LLAPs. Through this process the columns may also become contaminated with other environmental microorganisms. When using Qiagen QIAamp DNA minikit for extracting DNA for an *Aspergillus* PCR assay, we found that the columns and reagents were also frequently contaminated with fungal DNA (unpublished observations). Consequently, Qiagen columns at present may be unsuitable for the laboratory diagnosis of environmental microorganisms (especially those with a water habitat) and not just *Legionella* spp.

TABLE 1. Partial sequence alignment of 16S rRNA region indicating alignment of legionella contaminant with nearest matches in a BLAST search

Organism	Accession no.	Sequence alignment
L. lytica LLAP 3	X97358	ATCGGAATTACTGGGCGTAAAGAGTGCGTAGGTGGTTTGGTAAGTTATCTGTGAAATCCCTGGGCTCAACCTGGGCAG
LLAP 9	X97360	ATCGGAATTACTGGGCGTAAAGAGTGCGTAGGTGGTTTGGTAAGTTATCTGTGAAATCCCTGGGCTCAACCTGGGCAG
L. lytica strain L2	X97364	ATCGGAATTACTGGGCGTAAAGAGTGCGTAGGTGGTTTGGTAAGTTATCTGTGAAATCCCTGGGCTCAACCTGGGCAG
LLAP 7	X97365	ATCGGAATTACTGGGCGTAAAGAGTGCGTAGGTGGTTTGGTAAGTTATCTGTGAAATCCCTGGGCTCAACCTGGGCAG
LLAP 6	X97359	ATCGGAATTACTGGGCGTAAAGAGTGCGTAGGTGGTTTGGTAAGTTATCTGTGAAATCCCTGGGCTCAACCTGGGCAG
LLAP 2	X97356	ATCGGAATTACTGGGCGTAAAGAGTGCGTAGGTGGTTTGGTAAGTTATCTGTGAAATCCCTGGGCTCAACCTGGGCAG
LLAP 14	U66104	ATCGGAATTACTGGGCGTAAAGAGTGCGTAGGTGGTTTGGTAAGTTATCTGTGAAATCCCCGGGCTCAACCTGGGCAG
L. birminghamensis	Z49717	ATCGGAATTACTGGGCGTAAAGAGTGCGTAGGTGGTTTAGTAAGTTAACTGTGAAATCCCTGGGCTCAACCTGGGCAG
Legionella contaminant		ATCGGAATTACTGGGCGTAAAGGGTGCGTAGGTGGTTTGATAAGTTGTCTGTGAAATTCCTGGGCTCAACCTGGGCAT
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L. lytica LLAP 3	X97358	GTCAGATAATACTGCTGAACTCGAGTATGGGAGAGGGTAGTGGAATTTCCGGTGTAGCGGTGAAATGCGTAGAGATCGG
LLAP 9	X97360	GTCAGATAATACTGCTGAACTCGAGTATGGGAGAGGGTAGTGGAATTTCCGGTGTAGCGGTGAAATGCGTAGAGATCGG
L. lytica strain L2	X97364	GTCAGATAATACTGCTGAACTCGAGTATGGGAGAGGGTAGTGGAATTTCCGGTGTAGCGGTGAAATGCGTAGAGATCGG
LLAP 7	X97365	GTCAGATAATACTGCTGAACTCGAGTATGGGAGAGGGTAGTGGAATTTCCGGTGTAGCGGTGAAATGCGTAGAGATCGG
LLAP 6	X97359	GTCAGATAATACTGCTGAACTCGAGTATGGGAGAGGGTAGTGGAATTTCCGGTGTAGCGGTGAAATGCGTAGAGATCGG
LLAP 2	X97356	GTCAGATAATACTGCTGAACTCGAGTATGGGAGAGGGTAGTGGAATTTCCGGTGTAGCGGTGAAATGCGTAGAGATCGG
LLAP 14	U66104	GTCAGATAATACTGCTAGACTCGAGTATGGGAGAGGGTAGTGGAATTTCCGGTGTAGCGGTGAAATGCGTAGAGATCGG
L. birminghamensis	Z49717	GTCAGGTATGACTGTTAGACTAGAGTATGGGAGAGGGTAGTGGAATTTCCGGTGTAGCGGTGAAATGCGTAGAGATCGG
Legionella contaminant		GTCAGATGATACTGTTAGACTAGAGTATAGGAGAGGGTAGTGGAATTTCCGGTGTAGCGGTGAAATGCGTAGAGATCGG
		***** * **** * *** ****** **********
L. lytica LLAP 3	X97358	AAGGAACACCAGTGGCGAAGGCGGCTACCTGGCCTAATACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGAT
LLAP 9	X97360	AAGGAACACCAGTGGCGAAGGCGGCTACCTGGCCTAATACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGAT
L. lytica strain L2	X97364	AAGGAACACCAGTGGCGAAGGCGGCTACCTGGCCTAATACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGAT
LLAP 7	X97365	AAGGAACACCAGTGGCGAAGGCGGCTACCTGGCCTAATACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGAT
LLAP 6	X97359	AAGGAACACCAGTGGCGAAGGCGGCTACCTGGCCTAATACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGAT
LLAP 2	X97356	AAGGAACACCAGTGGCGAAGGCGGCTACCTGGCCTAATACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGAT
LLAP 14	U66104	AAGGAACACCAGTGGCGAAGGCGGCTACCTGGCCCAATACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGAT
L. birminghamensis	Z49717	AAGGAACACCAGTGGCGAAGGCGGCTACCTGGCCTAATACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGAT
Legionella contaminant		AAGGAACACCAGTGGCGAAGGCGGCTACCTGGCCTAATACTGACACTGAGGCACGAAAGCGTGGGGAGCAAACAGGAT
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