Precaution Using Nucleic Acid Based Methods to Detect *Aeromonas*

*Aeromonas* is ubiquitous in aquatic ecosystems but can cause various human infections including gastrointestinal tract syndromes and soft tissue infections (3). Its role as a diarrheal agent remains controversial but several studies show a substantial association (1, 2, 4, 6). For this reason we included *Aeromonas* in our recent multiplex PCR-Luminex assay for several bacterial enteropathogens (5). This letter is intended to urge caution when using such molecular methods to detect *Aeromonas*. In our work (5) we demonstrated the analytical performance of the PCR-Luminex assay using fecal samples that were spiked with relevant bacterial pathogens and extracted with QuickGene DNA Tissue kit (Fujifilm, Tokyo, Japan). However after press, when we subsequently used the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA), 71% (25/35) previously negative stool samples yielded low-level amplification of *Aeromonas* (e.g., qPCR Ct 30.7 to 37.4). This was confirmed with two distinct singleplex real-time PCR assays, one that used a Taqman probe to detect the same region of the aerolysin gene and another that used SYBR Green to detect an 16S rRNA region – primers and probes are published in (5). We dissected the Qiagen extraction components into 1) Elution buffer (AE) alone; 2) Wash buffers (AW1 and AW2) and Elution buffer; 3) Lysis buffer (AL) and ethanol, followed by Wash buffers and Elution buffer; 4) Stool Lysis buffer (ASL), followed by Lysis buffer, ethanol, Wash buffers, and Elution buffer; 5) InhibitEX tablets and Stool Lysis buffer, followed by Lysis buffer, ethanol, Wash buffers, and Elution buffer. These preparations were prepared in duplicate, DNA was extracted following the manufacturer’s instructions, and real-time PCR performed using both *Aeromonas* assays. Extracts from the first four procedures were negative (16/16) while the fifth one was positive (4/4). We then tested an additional 8 tablets of InhibitEX (from 4 lots) and all eight of these extracts were positive for *Aeromonas* (using both assays). These aerolysin
amplicons were sequenced and were 100% identical to Accession No. EF189591, sequence of an
*Aeromonas hydrophila* strain ZN1 distinct from our spiked *A. hydrophila* strain (28SA) with only
92% identity, arguing against contamination. We revisited the clinical samples that we evaluated
in our study (5). This revealed that 3/10 clinical specimens were low-level *Aeromonas* positive
(e.g., Ct > 30.7) contained the questionable ZN1 sequence. The corrected sensitivity/specificity
remained similar at 89%/95%. Our interpretation is that there may exist traces of DNA in certain
molecular kit components since *Aeromonas* is common to water. We do not know the extent of
*Aeromonas* detection using other extraction procedures, but we would urge one to pay special
attention to this step when trying to detect *Aeromonas* using molecular methods.

REFERENCES


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