Methods for the extraction of PCR-quality DNA from environmental soil samples by using pairs of commercially available kits were evaluated. Coxiella burnetii DNA was detected in spiked soil samples at <1,000 genome equivalents per gram of soil and in 12 (16.4%) of 73 environmental soil samples.
Qiagen stool kit was loaded with 700 µl of material (high volume). When a low volume of starting material was used, combinations of the two Qiagen kits also removed inhibitors from 100% of the samples when either the Qiagen tissue protocol was used first or the Qiagen stool protocol was used first (Fig. 1). The raw data for all of the inhibition assays are included as supplemental data (see Table S1 in the supplemental material).

To determine the yield of DNA obtained by the various protocols, nine aliquots (5 g each) of a single rich organic soil sample were each mixed with 5 ml PBS, spiked with 1 × 10^6 Nine Mile Phase 2 C. burnetii organisms, and then processed by the nine (high-volume) extraction protocols described above. An additional 1 × 10^6 Nine Mile Phase 2 C. burnetii organisms were used directly in the Qiagen tissue protocol to prepare DNA for the purpose of determining the exact amount of C. burnetii input into the assays. The quantitative IS1111 PCR assay (4) was used to determine the yield of C. burnetii DNA by using the various methods for processing soil. The yield was calculated by dividing the number of genome equivalents of C. burnetii DNA obtained from the spiked soil samples by the number of genome equivalents obtained when C. burnetii was included directly in the Qiagen tissue protocol. A common feature of all of the protocols was that they all produced a low yield of C. burnetii DNA when purified from a complex soil mixture (Fig. 2). The yields ranged from 0.02% to 4.3% and were variable. Although the 4.3% yield obtained when the stool kit was used alone was the highest on average, the high variability observed with these extractions suggests that most of these protocols provide similar yields. The stool kit followed by the MoBio kit clearly resulted in the lowest yield.

Although these yields are low, the IS1111 PCR assay used to detect C. burnetii DNA amplifies a multicopy gene, and the assay can detect a single genome equivalent (4). This suggests that these protocols are adequate for the detection of C. burnetii in soil samples with 500 to 2,000 organisms per gram of soil. To test this, a 5-g sample of organic soil was spiked with 800 C. burnetii organisms per gram, and the DNA was extracted using the MoBio UltraClean kit followed by the QIAamp stool protocol. C. burnetii DNA was detected after 38 cycles using the IS1111 PCR assay.

While these results are focused on soil samples, the procedures described also work well on vacuum samples and sponge wipe samples (data not shown). Based on removal of inhibitors and yield, our data suggest that the QIAamp tissue protocol (high volume) followed by the QIAamp stool protocol and the MoBio UltraClean kit followed by the QIAamp stool protocol are both suitable for extraction of DNA from environmental soil samples. To test the application of the latter method to a larger number of samples, 73 bulk soil samples from the southeastern United States were processed according to this method. Inhibition was removed from all 73 samples, and 12 of the samples were positive in the C. burnetii IS1111 PCR assay. This suggests that this practical method for extraction of PCR-quality DNA can be successfully used to detect DNA from C. burnetii and other pathogens in large numbers of environmental samples.

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REFERENCES


