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Contamination of Commercial PCR Master Mix with DNA from *Coxiella burnetii*[▽]

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Contamination of an in-house diagnostic real-time PCR for Q fever was traced back to a commercially obtained PCR Master Mix. It was established that this Master Mix contained DNA from *Coxiella burnetii*, probably as a result of the use of compounds of animal origin such as bovine serum albumin.

As of 2007 and up to the present time, there has been a large ongoing outbreak of Q fever in Netherlands that is unprecedented both in the number of affected individuals and in its duration (5). Real-time PCR has rapidly been implemented in many diagnostic laboratories in Netherlands as a first-line diagnostic test to identify patients infected with *Coxiella burnetii* in the acute phase of the disease. In a recent interlaboratory study, it has been shown that multiple PCR approaches based on different combinations of extraction procedures, amplification primers and probes, PCR Master Mixes, and real-time PCR platforms offer equivalent solutions to screening for Q fever (6). In the spring of 2009, the medical microbiology laboratory of the Canisius Wilhelmina Hospital (CWZ) was suddenly confronted with positive PCR results in a no-template control (NTC), indicating contamination of the diagnostic pathway. Analysis of multiple NTCs showed that 10 to 30% yielded a positive PCR result with threshold cycle values such as those found in many clinical samples. The laboratory enforces strict precautionary measures to avoid amplicon or DNA carryover during the entire diagnostic process. Since the contamination coincided with the first use of a new batch of PCR primers and probe, it was suspected that one of these was contaminated off synthesis. A newly ordered batch of primers and probe did not alleviate the problem. Around the same time, the medical microbiology laboratory of the Radboud University Nijmegen Medical Center (RUNMC) experienced similar problems, with important clinical consequences. A patient with a history of a Ross procedure was admitted with fever and probable endocarditis with gradual degradation of the aortic homograft and deterioration of cardiac function despite broad-spectrum antibiotics. All blood cultures were negative, and additional serological tests for culture-negative endocarditis (including Q fever) were performed. During this time, hemodynamic problems occurred and a second cardiac surgery was necessary. The day before surgery, the Q-fever PCR was positive but serological tests (complement fixation test and IgM antibodies) were negative, making chronic Q fever unlikely. It was decided to postpone the cardiac surgery and to repeat the PCR in another laboratory. This PCR was negative, confirming the suspicion of a false-positive PCR result. Fortunately, no serious hemodynamic problems occurred during the time while the operation was deferred. According to protocol and good laboratory practice, numerous measures were undertaken to eliminate any potential source of contamination, but without success. The laboratories of the CWZ and RUNMC used different PCR primers and probes targeting a different part of the *C. burnetii* specific IS*1111a* element, but both used the same commercially available PCR Master Mix. The presence of *C. burnetii* DNA in this Master Mix was then suspected. This could very well be the result of the presence of compounds of animal origin (such as bovine serum albumin [BSA]) that are commonly used in PCR assays. Since many farm animals can be carriers of *C. burnetii* (1, 4), addition of BSA originating from such animals may explain the contamination of the Master Mix with *C. burnetii* DNA. An unopened vial of this Master Mix was sent to the St. Antonius Hospital in Nieuwegein (a laboratory that routinely uses a different Master Mix; uses another, nonoverlapping, DNA target in its Q fever PCR; and had experienced no contamination issues). This again led to ~30% positive PCR results with NTCs, confirming the presence of *C. burnetii* DNA in the Master Mix. These findings were communicated to the manufacturer of the Master Mix, who initiated their own research and later confirmed the contamination of the Master Mix with DNA from *C. burnetii*. The manufacturer also notified users of the product of this contamination issue.

With the application of molecular techniques to detect microbial DNA in clinical samples, it has become apparent that there are many caveats at multiple stages in the diagnostic process that may lead to false-positive PCR results. Not only PCR enzymes may be contaminated with bacterial DNA (2) but in the DNA extraction procedures, reagents and disposables (such as spin columns) may also expose clinical samples to exogenous DNA (3). We report here the contamination of a commercially available PCR Master Mix with DNA from *C. burnetii*. This problem is probably related to the use of compounds of animal origin. Use of such compounds in PCR

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Master Mixes requires additional measures to ensure the absence of contaminating DNA. Problems similar to that reported here may be possible with other microorganisms that may be asymptomatically present in animals, such as *Campylobacter*, *Brucella*, *Listeria*, *Mycobacterium*, and *Toxoplasma*. It is highly unlikely that the problem described here is restricted to a single manufacturer of PCR Master Mixes. These findings highlight the requirement for extensive controls at multiple levels in PCR-based procedures to validate their use with clinical samples.

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