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SHORT COMMUNICATION

Presence of Murine Leukemia Virus (MLV)-related Virus Gene Sequences in a Commercial RT-PCR Reagent

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SUMMARY

Background: **The recent identification of murine leukemia virus (MLV)-related viruses in patients with chronic fatigue syndrome (CFS) has aroused much interest, not least among sufferers. However, other studies failed to detect these viruses in CFS patients.**
Methods: **We wanted to establish a MLV-related virus real-time PCR for routine diagnostics.**
Results: **Our study identified false positive MLV-related virus results due to a contamination of Superscript III Platinum One-Step Quantitative RT-PCR System (Invitrogen).**
Conclusions: **This observation may be helpful to elucidate discrepant results for the detection of MLV-related virus like xenotropic MLV-related virus (XMRV) in recently published studies.**
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KEY WORDS

MLV-related virus, XMRV, contamination, PCR-reagent, real-time PCR

INTRODUCTION

A recent study reported a high percentage of patients with chronic fatigue syndrome (CFS) to be infected with a murine leukemia virus (MLV)-related virus that has been designated xenotropic murine leukemia virus-related virus (XMRV) (1). More recently, another study detected MLV-related virus gene sequences in 32 of 37 (86 %) CFS patients compared with only 3 of 44 (7 %) healthy volunteer blood donors (2). The virus gene sequences in this study were more closely related to those of polytropic mouse endogenous retroviruses. However, other reports failed to detect XMRV or MLV-related virus sequences in CFS patients (3-6). We tried to establish a MLV-related virus detection system in our accredited Molecular Diagnostics Unit.

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MATERIALS AND METHODS

For this purpose a real-time PCR assay was developed with the previously described primer set NP116 (5'-CA TGGGACAGACCGTAACTACC-3') NP117 (5'-GCA GATCGGGACGGAGGTTG-3') (2) and the TaqMan probe (5'-FAM-CTGAGTCTAACCTTGCAGCACTG GGGAGAT-BBQ-3') (designed for this study) to amplify and visualize a 380 bp fragment of the gag gene. The reaction mixture contained 10 µL of 2X Superscript III Platinum One-Step Quantitative RT-PCR System Reaction Mix (Invitrogen, Lot.No. 640943), 0.4 µM of each primer, 0.1 µM of the probe, and 5 µL DNA or H_2O (LiChrosolv, Merck) in a reaction volume of 20 µL. Cycling conditions were 50 °C for 10 minutes, 95 °C for 2 minutes, followed by 45 cycles of 95 °C for 45 seconds, 55 °C for 45 seconds and 72 °C for 30 seconds using a LightCycler 480 instrument (Roche Diagnostics).
In the next step we evaluated a commercial RT-PCR reagent from another manufacturer. The real-Time PCR was performed as decribed above with the QuantiFast Probe RT-PCR Kit (QIAGEN, Lot.No. 136259134) instead of invitrogen chemistry. Sequencing of PCR-amplified fragments was performed for both strands using primers NP116 and NP117 with a BigDye terminator 3.1 kit (Applied Biosystems) according to the manufac-

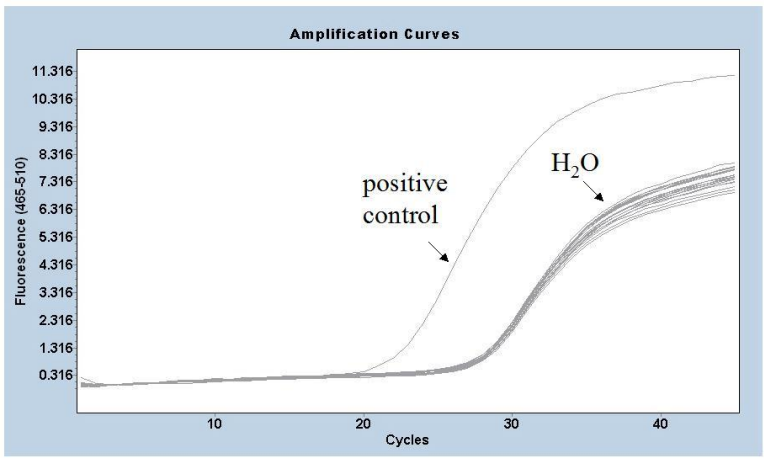

Figure 1. MLV-related virus real-time PCR with Superscript III Platinum One-Step Quantitative RT-PCR System Reaction Mix (Invitrogen): The positive control and all 20 H_2O controls were positive.

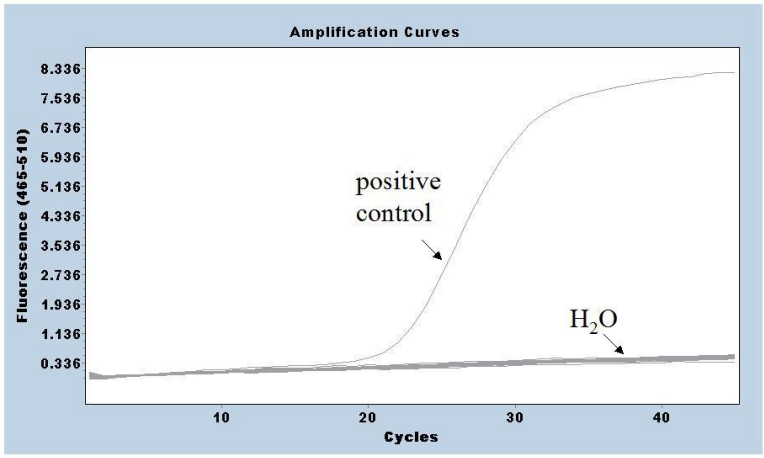

Figure 2. MLV-related virus real-time PCR with QuantiFast Probe RT-PCR Kit (QIAGEN): Only the positive control was positive. All 20 H_2O controls were negative.

turer's instructions. Capillary sequence analysis was performed with a 310 Genetic Analyzer (Applied Biosystems). The nucleotide sequences were compared with database entries by using the Blast program of the National Center for Biotechnology Information (NCBI).

RESULTS

3 randomly selected nucleic acid extracts from routine diagnostic blood samples, triplicate H_2O (negative) controls, and one positive control were included in the first RT-real-time PCR run with Invitrogen chemistry. The positive control was a dilution of a plasmid with a XMRV (isolate VP62) insert, generously gifted by Dr. N. Fischer (University Medical Center Hamburg-Eppendof). Surprisingly, all PCR reactions were positive in this run (data not shown). To confirm our observations a second run with 1 positive control and 20 H_2O controls was peformed. All 20 H_2O controls were positive (Figure 1). The results were confirmed in the 3 following runs with 10 H_2O controls each. The PCR products were visualised on a 2 % agarose gel stained with ethidium bromide. All PCR products revealed the predicted size of ~ 380 bp. We sequenced the PCR products of the positive control and 5 H_2O controls. As expected the sequence of the positive control matched perfectly with the XMRV isolate VP62 (GenBank: EF185282.1). All PCR-products of the H_2O controls revealed identical sequences most closely related (379 of 380 bp) to MLV-related virus (e.g. GenBank: HM630 560.1) which were also found in the study by Lo et al. (2). The sequences of the positive control and the H_2O controls differed in 12 of 380 bp. For this reason a contamination of the reaction mix with the positive control could be excluded. Additionally, no MLV had ever been cultured in our laboratory and no MLV vectors have been used so far.

In the next step, we evaluated a commercial RT-PCR reagent from QIAGEN. Again we performed a control run with 1 positive control and 20 H_2O controls. All 20 H_2O controls were negative (Figure 2). In the three following runs 70 of 70 H_2O controls were negative. Therefore we detected a contamination in the Invitrogen but not QIAGEN RT-PCR Reagents.

DISCUSSION

A number of studies have indicated the presence of microbial nucleic acids in PCR reagents (7-12). The contamination of a commercial RT-PCR kit with MLV-related virus was confirmed by a very recent study performed by Sato et al. (13). Probably, the contamination of the Invitrogen Kit is caused by a murine monoclonal antibody blocking the cold Taq polymerase acitivity. Murine hybridomas for manufacturing monoclonal antibodies also secret high amounts of retroviral particles which can cause the contamination (14). Two recent studies strongly suggest that MLV-related virus sequences in human patient samples originated from mouse DNA that contaminated the samples prior to PCR (15,16). If MLV-related virus detection in CFS patients resulted only from laboratory contamination, it is difficult to explain why the virus is detected more frequently in CFS samples than in healthy volunteer blood samples. Probably, besides the use of special PCR reagents preanalytical problems exist which can result in different amounts of contamination. Weiss (14) suspected it may be that the vials containing CFS specimens tend to be handled more often than simple healthy blood samples. Future studies are necessary for this important issue.

Furthermore, it is important to note that, while our observations demonstrated the prevalence of contamination by MLV-related virus gene sequences in the One-Step Quantitative RT-PCR System from Invitrogen, it does not exclude the possibility of contamination in the reagents of other companies. The identificaction of MLV-related virus gene sequence contamination in RT-PCR reagents should be relevant for the scientific community.

PCR reagents, especially with only low amounts of contamination, can produce artificial results that can be extremely confusing and misleading. We consider large-scale tests for contamination of great importance before investigating XMRV or MLV related disease in clinical specimens.

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Declaration of Interest:
The authors report no conflict of interests.

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