Introduction
Two-stage revision arthroplasty is recognized as a standard therapy in known infected arthroplasties. During the first operation, the infected material is removed, and an antibiotic-containing spacer composed of polymethylmethacrylate (PMMA) is placed. Some studies have shown that bacteria are capable of adhering to bone cement and of forming biofilms in vitro. Therefore it is important to detect biofilm-formative bacteria associated with bone cement. The sensitivity of diagnostic assays has been improved by the use of techniques that dissolve adherent bacteria from the biofilm. Ultrasonication and/or vortexing have been shown to increase the number of bacteria isolated from retrieved joint implants. The aim of this study was to evaluate the effectiveness of vortexing (V) and ultrasonication (US) to detect biofilm-formative bacteria from PMMA by conventional quantitative culture and real-time quantitative polymerase chain reaction (qPCR).

Materials and Methods
Bacteria and culture: Biofilm formative Staphylococcus aureus (S. aureus ATCC 12600) was used in the in vitro study. We used 20 PMMA coupons (Stryker Orthopaedics, Mahwah, NJ; diameter: 26 mm, thickness: 3 mm) without antibiotics. Each coupon was placed in 10 mL Tryptic soy broth medium (TSB) containing 1.5 x 10^8 colony-forming units (CFU)/mL of biofilm-formative S. aureus and incubated initially for 4 hours. The coupons were washed once with 25 mL phosphate-buffered saline (PBS), transferred to a sterile container with fresh TSB broth, and re-incubated for 15 hours to allow biofilm formation. The coupons were then washed twice with 25 mL of PBS to remove any non-adherent bacteria from the surface, and transferred to a new sterile cup with 25 mL PBS. The coupons were vortexed for 30 seconds inside the cup (Maxi Mix II, Barnstead/International, Dubuque, IA) and then subjected to ultrasonication at a frequency of 40 kHz (Branson Ultrasonic Cleaner; Branson Ultrasonics, Danbury, CT) for different lengths of time, followed by additional vortexing for 30 seconds (vortexing and ultrasonication = V+US+ groups). Control groups were coupons that were neither vortexed nor sonicated (V-US- group), and coupons that were vortexed but not sonicated (V+US- group). Three different durations of sonication were examined: 1, 5, and 30 minutes.

Discussion
Vortexing is a convenient, inexpensive, widely available technique, and is effective in dislodging bacteria adherent to bone cement. Because we did not observe any differences among 1, 5, and 30 minutes of sonication, a sonication time around 1 minute may be practical for clinical use. The techniques of vortexing with or without ultrasonication could be easily introduced into the routine microbiologic laboratory. However, care must be taken when interpreting the current information for clinical use because compared with our in vitro results, bone cements retrieved from patients may include different bacteria, different duration of infections, and variability in antibiotic load and antibiotic susceptibility of the bacteria. We recommend using these simple methods in prospective clinical studies intended to detect the frequency of persistent infection in patients undergoing the second stage of re-revision arthroplasty for a known infection. The methods may also be used to help recognize infection in patients thought to have experienced aseptic loosening of cemented total hip and knee implants.

References