Performance of the Unyvero i60 System with Sonication Fluid

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Identification of the causative pathogen and its antibiotic resistances is essential for the successful treatment of patients with infections of orthopedic implants. Sonication fluid as sample material has been used to determine pathogenic bacteria in biofilms on orthopedic implants. With the sonication protocol described, the Unyvero i60 tests identified the same pathogens as microbial culture in the majority of the cases. Additionally, the Unyvero i60 test identified 7 pathogens within 40 samples that were missed by microbial culture.

Orthopedic implants are used for the treatment of degenerative joint disease as osteoarthritis and bone fracture¹. In Germany, 232,612 patients received a hip replacement and 168,536 patients received a total knee replacement in 2011². The number of periprosthetic joint infection is increasing due to higher patient number and increased resident time of the implant in the body. These implants can become infected via the haematogenic route after implantation³,⁴. In Germany, 25,807 patients received a secondary hip replacement in 2011⁵. Identification of the causative pathogen is a critical factor for the therapy guidance and decision in successful treatment of prosthetic joint infection. Samples for microbial investigation can include pus, synovial fluid, soft tissue, bone and prosthetic components⁶. Cultures of sinus tract exudates are often positive due to skin flora and intraoperative swab cultures have low sensitivity and should be avoided⁷. To improve diagnosis of prosthetic joint infection, multiple tissue samples in proximity to the prosthesis should be taken⁸. Sonication of explanted prosthesis can improve the diagnosis of periprosthetic joint infection⁹,¹⁰ of hip and knee¹¹ or shoulder¹².

Material and Methods

Sonication fluid was collected and analyzed by standard microbial culture. 40 Sonication fluids were collected. Routine microbiology identified ≥1 significant pathogen in 12 samples with 50 – 10,000 CFU, in 8 additional samples growth was only found after enrichment, 20 produced no growth.

Sonication fluid was collected and processed as follows:

Specimen
Components from prosthetic joint athroplasty or other implanted devices are aseptically placed into a sterile, sealable container. The following containers are suggested
- 1 L Straight-Side wide mouth jar, polypropylene
- 2.2 L Jar with snap top cover, polypropylene
- other sterile boxes with sealed cover, polypropylene

Note: The use of plastic bags for sonication is not recommended due to increased risk of contamination¹³.

Reagents/Supplies
- Sterile 0.9% NaCl, Ringer's salt solution or PBS, pH 7.4
- Centrifugation Tubes 50 ml
- Micro Reaction tubes
- Pipett Serological 1, 2, 50 ml
- Micro pipette for 180 µl

Equipment
- Ultrasonicator, Brand, Model, Volume, Frequency
  - Bandelin BactoSonic (40 kHz, max 200 W) power density 0.22W/cm²
  - Bransonic SM25E-M (40± 5 kHz)
- Aquasonic Model 750T ultrasound bath (VWR Scientific Products) (frequency, 40±2 kHz; and power density 0.22±0.04 W/cm²
- Centrifuge
- Pipetman
- Vortex

Procedure
1. Fill the sonicator to the marked line with deionized water from tap if needed.
2. Allow the sonication fluid to come to room temperature.
3. In the hood, pour the fluid into the container aseptically until the sample is completely covered or at least >90% covered with fluid, and close the cap.
4. Vortex the components in the container for 30 seconds at full power or shake it vigorously by hand. Hold on to the container sides to minimize splashing.
5. Sonicate the components in the plastic container for 1 - 5 minutes (depending on the size of the specimen at 100% (40 kHz).
6. Wipe excess water from outside of container and vortex the components in the container for 30 seconds at full power or shake it vigorously by hand. Hold on to
the container sides to minimize splashing.
7. Use 50 mL pipette to aliquot 50 mL sonicate into a conical 50 mL tube.
8. Centrifuge the 50 mL tube at 5000 rpm (3950 x g) for 20 minutes.
9. In the hood, remove the supernatant, keeping about 0.500 mL of sediment in the bottom of each tube.
10. Gently mix the sediment.
11. Use 180 µl of resuspended sediment for analysis in the UNYVERO.

Results
Use of a modified sonication protocol together with the Unyvero i60 application showed an excellent correlation between microbial culture and molecular test results. In 4 of 40 Sonication fluids pathogens were found, that are not part of the i60 panel (3x Ralstonia picketti and Morganella morganii). The i60 test identified the same pathogen as routine microbiology in 9/12 of the specimens showing growth in microbial culture. The missed organisms were Streptococcus mitis as well as the Ralstonia picketti and Morganella morganii that are not part of the measurement panel. In 5 samples where microbial culture identified a pathogen within the specimens, additional pathogen species were identified by the i60 test (4x Acinetobacter baumannii, 2x Staphylococcus aureus, Enterococcus sp.). In the 2/20 specimens where standard culture identified no growth, the i60 test found at least one pathogenic organism (Acinetobacter baumannii and Staphylococcus aureus). In the 2/8 samples, where microbial culture produced growth only after enrichment, the correct pathogens were found by the i60 system.

Discussion
When using sonication fluid as sample material for the Unyvero i60 test together with the suggested protocol, a very good correlation with microbial culture is achieved for the specimens showing growth in microbial culture and in the cases showing no growth in microbial culture. Additional pathogens could be found with the i60 test in these samples. Correlation with microbial culture was weak in these cases, where culture produced growth only following enrichment.

References