Supplementary Material

Sample Processing and DNA Extraction

For synovial fluid or prosthetic sonicate fluid, a 1.5mL microcentrifuge tube with 0.7mL lysis buffer and 0.6 mL sample and 1g 0.5mm glass bead was attached to a horizontal platform on a vortex mixer and agitated vigorously at 2,800 rpm to 3,200 rpm for 30 min. Total genomic DNA was extracted from samples using the TIANamp Micro DNA Kit (DP316, TIANGEN BIOTECH) according to the manufacturer's instructions. For periprosthetic tissue, 1.5mL microcentrifuge tube with 0.7mL lysis buffer and pieces of tissue sample and 1g 0.5mm glass bead were attached to a horizontal platform on a vortex mixer and agitated vigorously at 2800 3200RPM for 30 min. 0.3mL sample was separated into a new 1.5mL microcentrifuge tube and DNA was extracted using the TIANamp Micro DNA Kit (DP316, TIANGEN BIOTECH) according to the manufacturer's instructions.

Construction of DNA libraries

DNA libraries were constructed through DNA-fragmentation, end-repair, adapter-ligation and PCR amplification. Agilent 2100 was used for quality control of the DNA libraries. Quality qualified libraries were sequenced by BGISEQ-50 platform.

Sequencing and bioinformatic analysis

This process was conducted in the following manner: High-quality sequencing data were generated by filtering out the low-quality ($Q5 \leq 0.7$), and short (length < 35bp) reads, followed by computational substraction of human host sequences mapped to the human reference genome (hg19) using Burrows-Wheeler Alignment. The remaining data by removal of low-complexity reads were classified by simultaneously aligning to four Microbial Genome Databases, consisting of viruses, bacteria, fungi, and parasites. The classification reference databases were downloaded from NCBI(ftp://ftp.ncbi.nlm.nih.gov/genomes/). RefSeq contains 4,061 whole genome sequence of viral taxa, 2,473 bacteral genomes or scaffolds, 199 fungi related to human infection, and 135 parasites associated with human diseases.

Criteria for a Positive mNGS result:

As shown in Supplementary Figure 1

Bacteria (mycobacteria excluded): mNGS identified the organisms (species level) whose coverage rate scored 10 times or more than that of any other organisms. If not, and the organism was not a contaminant genus in negative control, mNGS identified organisms (species level) whose reads were more than 10. If the organisms were contaminant bacteria in the negative control and coverage on alignment to reference genome rate was more 2%, they were considered positive. mNGS identified organisms (genus level) whose reads more than 10 if the organism was not a contaminant genus in negative control. If the organisms were contaminant bacteria in the negative control and had been identified in species level, they were considered positive.

Fungus and Virus: mNGS identified the organisms (genus-level or species-level) which reads more than 10 due to the low biomass of these organisms in DNA extraction.

Mycobacteria: For Mycobacterium tuberculosis (MTB), due to the difficulty of DNA extraction and low possibility for contamination, when at least 1 taxon-specific read was mapped to either the species or genus level, the result will be considered as MTB positive. Nontuberculous mycobacteria (NTM) were defined as positive when the mapping read number (genus or species level) was in the top 10 in the bacteria list due to the balance of hospital-to-laboratory environmental contamination and low yield rate.

Parasites are not considered.

Supplementary Figure 1 . Criteria for a Positive mNGS result