Supplementary Appendix 2

Details of Methodology in regard to Ibis T-5000 Universal Biosensor Analysis

Introduction:
The Ibis T-5000 Universal Biosensor technology[7] uses a PCR-ESI-TOF MS approach which gives the exact base composition for each amplimer generated. Since multiple (8-16) amplimers are generated for each assay it is possible using a comprehensive database to provide a list of possible species matches for each amplimer. A triangulation approach is used in which all of the amplimers are considered together to arrive at a definitive species-level diagnostic for all known bacteria. In addition, the system provides a most-closely-related match for unknown organisms. The concordance for non-rare taxa between the Ibis analyses and deep 16S sequencing is extremely high between the two methods[18]. The Ibis system, however, is not effective at picking up rare taxa, present at less than 1-3% of the total microbial burden, with the exception of *Staphylococcus aureus* which has its own specific primer. The advantage of this technology was that it freed us from having to choose what organism to test for as there is no need for an a priori hypothesis as to what species are present.

Summary of Methodology:
Total DNA was extracted from all urine samples, and microbial (i.e., bacterial and fungal) DNAs were amplified by the polymerase chain reaction (PCR) using the 16 primer pair BAC (Bacteria, antibiotic resistance genes and Candida) and Fungal detection systems developed by Ibis as described[7]. The individual amplicons were "weighed" using the Ibis instrumentation by electrospray ionization (ESI) time-of-flight (TOF) mass spectrometer (MS) which reports out the molecular mass. The amplicon masses are then used to determine their base compositions as a particular mass can only be produced by a single combination of the four nucleotides (A, C, G, & T). The taxonomic identities of the amplicons were then revealed using a database[7] containing base composition data on virtually all bacterial/fungal species sequenced to date. Details of the methodology is available in Appendix.

Details:
For all samples, 3 ml of urine were centrifuged at 10,000 rpm x 3 min, then all but 100 µL of supernatant was removed and remaining pellet added to 270 µL ATL lysis buffer and proteinase K. Nucleic acid from the lysed sample was extracted using the Qiagen DNeasy Tissue kit (Qiagen cat# 69506). 10 µL (total elution volume of 200 µL) of each sample was then loaded per well onto the Ibis BAC/Fungal detection PCR plates (Abbott Molecular; Abbott Park, IL). Each detection plate is comprised of 96 wells containing 16 primer pairs per assay that survey all bacterial (as well as Candida and related yeast species) organisms through a combination of: (1) omnipresent loci (e.g. 16S/18S rDNA sequences); (2) phylum/class/order specific loci; and (3) target loci for specific pathogens of interest (e.g. the *Staphylococcus*-specific *tuf*B gene). PCR amplification was carried out as recommended by the manufacturer followed by desalting of the PCR products, which were then sequentially electrosprayed into the TOF MS. The spectral signals were then processed to determine the masses of each of the PCR products present with sufficient accuracy that the base composition of each amplicon could be unambiguously deduced. Using combined base compositions from multiple PCRs, the identities of the pathogens and a semi-quantitative determination of their relative concentrations in the starting samples was established using a proprietary algorithm to interface with the Ibis database of known organisms[26]. The BAC system also detects the presence of several key antibiotic resistance markers, including van A and van B (vancomycin resistance) in *Enterococcus* species, KPC (carbapenem resistance) in Gram-negative bacteria, and mec A (methicillin resistance) in *Staphylococcus* species. An internal calibrant consisting of a known amount of a synthetic nucleic acid template is also included for each primer pair in each assay, controlling for false negatives (e.g. from PCR inhibitors) and enabling the semi-quantitative analysis of the amount of template DNA present.

Identification of Microbial Species:
This PCR-ESI-TOF MS approach gives the exact base composition for each amplimer generated. Since multiple (8-16) amplimers are generated for each assay it is possible using a comprehensive database to provide a list of possible species matches for each amplimer. A triangulation approach is used in which all of the amplimers are assessed in combination to generate a definitive species-level diagnostic for all known bacterial species. In

addition, the system provides a “most-closely-related match” for unknown organisms. This technology allowed for a powerful discovery-based approach that was not subject to restrictions based on a priori assumptions of microbial profiles.