No anti-SARS-CoV-2 nucleocapsid protein IgG antibody was detected in any of the vaccinated or healthy participants, indicating that the anti-SARS-CoV-2 S1 IgG assay is specific for the mRNA vaccine-induced antibodies. **Conclusions:** This method can be used for quantitative detection and monitoring of post-vaccination anti-SARS-CoV-2 spike IgG responses.

ID041. **Validation of Whole-Genome Sequencing of Bacterial Isolate**

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Introduction: The incidence of hard-to-treat bacterial infections is increasing in hospitals worldwide and enhancing the central role of rapid and accurate identification of the agent, for timely and effective treatment for the patients. The bacterial whole-genome sequencing (WGS) technique is becoming widely used in clinical diagnostic, public health laboratories, and in research. This technique can help in accurate identification of the causative bacterial pathogen, and can potentially evaluate virulence and antibiotic resistance, as well as providing data for molecular epidemiology in outbreaks, by clonality analysis. **Methods:** We selected 34 bacteria strains of different species previously identified by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF). Seven of the 34 samples were also submitted to antimicrobial susceptibility test (AST) in the VITEK2 AST cards (Cards for Antimicrobial Susceptibility Testing). For reproducibility, we selected three samples for triplicate intra-assay and three samples for triplicate inter-assays. DNA from isolated cultures was extracted and used on library preparation for next-generation sequencing (NGS) using 300pb paired end sequencing run. For data analysis, the genome taxonomy was assigned using GTDB-Tk v2.1.0. The mean nucleotide identity (ANI) between the genome and the closest reference was determined using FastANI. Resistance-encoding mutations were predicted using the Comprehensive Antibiotic Resistance Database (CARD) Resistance Gene Identifier (RGI) v5.1.1. **Results:** The sequencing quality metrics were high satisfactory. All the strain presented a genomic coverage of at least 30x and mean of 98.8% and 0.93 for the ANI and alignment fractions parameters, respectively. We found correlation of 91% (31/34 samples) comparing the species identification by conventional MALDI-TOF and NGS, and 03 samples presented different species due to database update. Regarding resistance in the AST method, six strains presented resistance to imipenem and meropenem (carbapenems antibiotics class) and one strain only to imipenem. The WGS method was able to identify in the seven samples the antimicrobial resistance gene *NDM*-New Delhi metallo-β-lactamase (five samples NDM-1, one sample NDM-5 and one sample NDM-7) that is responsible in hydrolyzing a wide range of β-lactam antibiotics, including carbapenem, agreeing with the AST results. Intra- and inter-assay reproducibility showed 100% correlation between replicates. **Conclusions:** The WGS for bacterial isolate characterization demonstrated a high performance in the validation. The use of this technique in addition to conventional methods can help to improve the diagnostic of hard-to-treat infections worldwide.

ID042. **Mutation Analysis in Bedaquiline, Delamanid and Linezolid of Rifampicin Resistant Mycobacterium Tuberculosis in Korea Using WGS**

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Introduction: We analyzed 8,943 isolates on the Lowenstein-Jensen (L-J) phenotypic DST from January 2018 to June 2018 and 16,454 isolates on the L-J phenotypic DST from April 2020 to August 2021, a total of total 25,397 isolates in Korea. Rifampicin resistant (R-R) isolates were categorized according to MICs using 7H9 broth microdilution method (7H9 BMD). Bedaquiline (BDQ) showed 82.16% of ≤0.03125 ~0.125 μg/mL area, delamanid (DLM) showed 88.78% of ≤0.00625 ~0.0125 μg/mL area, together with a relatively large distribution (8.03%) of 0.4 ~0.8 μg/mL area despite its high concentration. The aim of this study is to analyze mutations associated with high MIC cases using whole-genome sequencing (WGS). **Methods:** We selected 101 R-R isolates with high BDQ, DLM, or linezolid (LZD) MICs among 25,397 isolates. We tested 101 isolates using Miseq benchtop sequencer (Illumina, Inc., San Diego, CA) for WGS. We analyzed the *atpE, mmpR* and *pepQ* regions for 32 isolates that were >0.25 μg/mL in BDQ. For DLM, we analyzed the *ddn, fgd1, fbiA, fbiB,* and *fbiC* regions for 48 isolates which were >0.2 μg/mL. For LZD, we analyzed *rrl* and *rplC* regions for 11 isolates which were >1.0 μg/mL. Additionally, we analyzed 15 isolates of ≤0.25 μg/mL in BDQ, ≤0.2 μg/mL in DLM, and ≤1.0 μg/mL in LZD. **Results:** Among 33 isolates, 27 (84.38%) had mutations in *mmpR (Rv0678)* for BDQ. We found correlation between phenotypic DST and WGS. We were unable to find the mutant types (MT) in *atpE* and *pepQ*. For DLM, 41/48 (85.41%) isolates were of MT in *ddn*. For LZD, 10/11 (90%) isolates were found to be of MT in *rplC* region. Among low MIC isolates, 2/15 (13.33%) isolates were of silent MT in *rplC* and *fbiA*. In this study, we were able to find mutations for BDQ, DLM, and LZD in 96 isolates from 2020 to 2021, but we were unable to find the mutation for BDQ and DLM, and only found *rplC* mutation for LZD in five isolates in 2018. **Conclusions:** By matching MIC results and WGS, we found that *mmpR*, *ddn,* and *rplC* mutations are correlated with high MICs of new drugs. However, in this study, only five isolates in 2018 were used to analyze WGS, Further investigation is necessary to analyze large numbers of isolates and confirm the mutations associated with resistance.

ID043. **Deciphering the Microbiology of Respiratory Infection Using Precision Metagenomics Analysis**

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Introduction: Metagenomic sequencing using next-generation sequencing (NGS) technology has unparalleled power of comprehensive microbial profiling. NGS can detect unknown target organism(s) often undetected by routine microbiological and polymerase chain reaction (PCR) methods. We deployed a comprehensive NGS panel for simultaneous detection of 187 bacteria, 42 viruses, 53 fungi, and 1,218 antimicrobial resistance markers in 20 respiratory infection samples. NGS results were compared to a 36-target PCR panel commonly used for molecular diagnosis of respiratory infection. **Methods:** DNA and RNA were extracted separately and we prepared libraries using the Illumina RNA Prep with Enrichment kit. Indexed libraries were enriched for microbial content by hybridization capture with the Respiratory Pathogen ID-AMR panel (Illumina). Final libraries were sequenced using the Illumina MiniSeq instrument to yield paired-end reads of 75 bp length. Sequencing data were analyzed by the IDbyDNA-Explify portal. A final explify report was generated containing the quantitative identification of viruses, bacteria, and fungi in each sample, including the antibiotic resistance marker. Same samples were also analyzed with a 36-target semi-quantitative PCR panel, and results obtained from both technologies were compared. **Results:** All the organisms identified by the PCR panel were likewise detected in microbiome analysis. Three bacteria and 10 viruses were commonly detected by both PCR and NGS with *Streptococcus pneumoniae, Moraxella catarrhalis,* influenza A virus, human rhinovirus, and human metapneumoviruses most common concurrently detected organisms. However, the PCR panel only identified the co-infection in 2/20 samples, whereas the NGS panel detected a minimum of three and up to 20 organisms in a single sample. Additionally, 25 organisms (three viruses, 20 bacteria, and two fungi) were exclusively identified by the microbiome analysis; the most

prevalent – not targeted on PCR – were *Elizabethkingia species* (12/20) and *Dolosigranulum pigrum* (9/20). Furthermore, *Moraxella catarrhalis* was detected in only 3/20 samples by PCR compared to 9/20 samples by NGS. **Conclusions:** Although PCR testing for respiratory pathogen detection remains a valuable tool, concurrent NGS analysis suggests that the etiological restriction of PCR testing may have clinical limitations. The NGS-based microbiome analysis revealed a much more comprehensive microbiology profile. Moreover, NGS-based analysis has the potential to improve clinical outcomes in complicated co-infection cases. NGS results can also be used for improving PCR panels by adjusting the target organism(s) according to clinical significance. Further investigations are required to establish the clinical utility of the precision metagenomic analysis using NGS assays.

ID044. **WITHDRAWN**

ID045. **The Vaginal Microbiome: Its Composition and Function in Three Countries**

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Introduction: The healthy vaginal microbiome is a unique community that tends to be dominated by a single species of *lactobacilli*; *L. crispatus, L. jensenii, L. iners,* or *L. gasseri.* A fifth healthy community exists that is non-*lactobacilli* dominated and has much higher diversity. These are termed community state types (CSTs) and they may be associated with risk of vaginal infection. CST I, the *L. crispatus*-dominated community, seems to be the most protective, followed by CSTs II (*L. gasseri*) and V (*L. jensenii*). CST III is dominated by *L. iners*, which displays fewer protective characteristics. Finally, CST IV, the diverse, polymicrobial community, may be least protective and most prone to infections such as bacterial vaginosis (BV). BV-affected women often display a dominance of *Gardnerella vaginalis* in their vaginal microbiota but the presence of this species does not always indicate that the disease is present. Regardless, detection of these microbiota and classification into CSTs could help physicians predict a woman's chance of vaginal infection and lead to closer monitoring and earlier treatment. **Methods:** In this work we compare the vaginal microbiota of women from three different countries: groups from Sweden (N = 45), China (N = 35), and two groups from the US (N = 51 and N = 30). We imported this whole-genome sequencing data from publicly available studies and performed a comparative microbiome taxonomic and functional analysis using the CosmosID-HUB. We performed alpha diversity, beta diversity, and differential abundance analyses for the taxonomic and functional data on the HUB. **Results:** We found every CST within each group and the samples clustered according to CST rather than country, implying a broad applicability of the CST classification system. However, when we predicted functionality of the microbial communities we found that they clustered by country rather than CST. This suggests that vaginal community functionality may be influenced by location, regardless of CST. Community functionality may be influenced by ethnicity, lifestyle practices, diet, or some other environmental factor. One functional group consisted of samples with enriched abundance of genes coding for mucin degradation, which has been previously associated with pathogenic potential. The women in this group were from a mix of the three countries. **Conclusions:** These results suggest that, in a healthy state, CSTs are globally present but their functionality may be influenced by location-specific factors. However, there may be a subset of functions with greater pathogenic potential that is not location-associated. These women may be more prone to vaginal infections, and their identification may aid physicians in catching and treating vaginal disease early on.

ID046. **Evaluation of Aptima HIV-1 Quant Dx Assay on the Panther System in a Pediatric Hospital**

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Introduction: Rapid and accurate diagnosis of human immunodeficiency virus type 1 (HIV-1) infection is critical for patient management, reduction of mortality, and prevention of forward transmission. The Aptima HIV-1 Quant Dx Assay (Aptima) (Hologic, Inc., San Diego, CA) is an *in vitro* nucleic acid amplification test for both the diagnosis of HIV-1 infection and quantitation of viral RNA in plasma samples on the fully automated Panther System (Hologic) in <4 hours. The assay quantitates HIV-1 RNA between 30 to 10 million copies/mL. Target capture, target amplification by transcription-mediated amplification, and detection of the amplicon by fluorescent labeled probes (torches) occurs in a single tube within the fully automated system. **Methods:** A total of 199 plasma samples were included in the study. Thirty-four were previously quantitated samples obtained from BioCollections Worldwide, Inc. (BWI), (Miami, FL). One hundred and thirty-one prospective and retrospective patient plasma samples, along with 34 contrived samples, previously tested at Texas Children's Hospital (TCH) by either the cobas AmpliPrep/cobas TaqMan HIV-1 Test (cobas) (Roche Diagnostics, Indianapolis, IN) and/or the APTIMA HIV-1 RNA Qualitative Assay (Hologic, Inc., San Diego, CA). To assess the accuracy of testing low volume plasma samples, a total of 43 samples (33 patient plasma samples and 10 contrived samples) originally tested by BWI and/or TCH using the cobas were diluted in HIV-1-negative normal human plasma and tested at TCH using the Aptima assay on each Panther system. All retrospective samples remained frozen until retested on the Panther using the Aptima assay. **Results:** The overall sensitivity of the Aptima assay for HIV-1 RNA detection was 92% (113/123). Ten samples with original results ranging from <20 copies/mL to 608 copies/mL were not detected by the Aptima assay. These discrepancies are likely due to sample degradation after multiple freeze-thaws or target loss from low level of viral content. Linearity of diluted and undiluted samples were analyzed by simple linear regression and showed correlation with R^2 values of 0.8867 and 0.8871 respectively. Limit of detection studies included dilutions of the SeraCare AccuSpan HIV-1 RNA Linearity Panel member 6 (viral load 2,014 copies/mL) tested in 10 replicates at 30 copies/mL, which demonstrated detectable HIV-1 RNA at 100% (10/10). Analytical specificity and precision/reproducibility were both 100%. **Conclusions:** The Aptima assay on the Panther system is a relatively easy-to-perform, sample-to-answer test with minimal hands-on time. The assay delivers sensitive and reliable results which enable early diagnosis of HIV-1 infection and quantitative information for patient staging and monitoring.

ID047. **Development of a Novel Triplex Reverse Transcription Loop Mediated Amplification (RT-LAMP) Assay**

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Introduction: Most loop-mediated isothermal amplification (LAMP)-based diagnostic methods are generally used for the detection of a single target per reaction, as current methods of detection in a LAMP assay are not sequence-specific and lack in differentiation among multiple targets amplified in a single reaction. To overcome this challenge, we have developed a novel method for detection of three targets in a single LAMP reaction using labeled primers. **Methods:** Triplex RT-LAMP assay was developed for detection of foot-and-mouth disease virus (FMDv) in cattle and pigs. LAMP primers targeting two different regions of FMDv were designed. For internal control (IC), LAMP primers were designed targeting a conserved region of the 18S rRNA gene. Primer designs were evaluated for specificity and sensitivity in an RT-LAMP assay. Each target (FMDv-1, FMDv-2, and 18S rRNA) was detected in a separate RT-LAMP assay, and reactions