2024 Young Investigator Award Winning Abstracts

ID020. **Housekeeping Gene Specimen Adequacy Controls Are Poor Proxies for Nasopharyngeal Swab Collection Quality**

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Introduction: Nasopharyngeal (NP) swabs are commonly used to test for respiratory virus infections and require trained personnel for proper collection. During the COVID-19 pandemic, nasal swabs were frequently self-collected. This improved access to testing but decreased true sampling of the nasopharynx, and likely increased variability in collection quality. Throughout clinical microbiology, increased use of self-collected specimens has driven interest in including internal housekeeping gene controls in qPCR assays to monitor specimen quality. However, there is still limited data on the utility and interpretation of these controls. To assess how NP swab collection quality impacts specimen adequacy internal control results, we conducted a clinical study examining qPCR results for 2 commonly used human housekeeping genes—ribonuclease P protein subunit p30 (*RPP30*) and beta-globin (*HBB*)—from NP swabs collected under pre-specified conditions. **Methods:** With IRB approval and written informed consent, NP swabs were collected from 20 participants from both nares or a single naris with good, suboptimal, or poor collection quality. Samples were collected from the nasopharynx for 5 seconds ("good") or 1 swab rotation ("suboptimal") or from the nostril only without swab rotation ("poor"). Forty NP swabs were collected for each condition for a total of 240 swabs. After collection, the swabs were frozen (≤-70°C) prior to qPCR testing at the University of Washington Virology Laboratory. Total nucleic acid was extracted using the MagnaPure 96 platform followed by *RPP30* or *HBB* qPCR. The *RPP30* assay includes a reverse transcription step and measures mRNA and DNA resulting in lower cycle threshold (Ct) values than for the *HBB* assay that only detects DNA. **Results:** *RPP30* was detected in ≥95% and *HBB* in ≥90% of samples from each condition. There was no significant difference in the ability to qualitatively detect the tested housekeeping genes based on sample collection method (Chi-squared test, *RPP30*: $p = 0.63$; *HBB*: $p = 0.93$). Excluding undetected samples, samples collected from both nares with good quality on average had significantly lower Ct values than samples with a poor collection method from one naris (*RPP30*: 27.2 versus 29.3, $p < 0.001$; *HBB*: 29.5 versus 31.3, $p < 0.001$). However, there was substantial overlap in the Ct value distribution for all conditions, preventing definitive assignment of collection quality by Ct value. **Conclusions:** Overall, our data show that specimen adequacy control qPCR results are only minimally representative of NP swab collection quality. Our study is unique for isolating sample collection quality as a variable for clinical study and highlights the complicated nature of interpreting specimen adequacy controls.

ID024. **Molecular Alterations in Azole-Resistant *Aspergillus Fumigatus***

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Introduction: *Aspergillus fumigatus* remains one of the most common fungal species in opportunistic infections, including invasive aspergillosis. The emergence of azole-resistant *Aspergillus fumigatus* has become a global health problem. Azole-resistant strains of *Aspergillus fumigatus* are known to be associated with mutations in *CYP51A* as well as *HAPE, HMG1,* and *HMG2*, among others.
The *HMG2* p.I235S alteration was previously thought to be involved in azole resistance but was recently reported in 1 azole susceptible strain. Here we performed whole-genome sequencing on azole-resistant isolates to characterize existing and novel molecular alterations associated with resistance. **Methods:** In this study, we performed whole-genome sequencing of 87 *A. fumigatus* isolates (80 passed quality control) selected from the UT Health Fungus Testing Laboratory (FTL) collection (years 2007 through 2022), which were submitted for susceptibility testing at the FTL. DNA was extracted using the EZ1 DNA tissue kit and whole-genome libraries were prepared using the KAPA HyperPlus Kit. The normalized diluted library pool was paired-end sequenced (2x160 PE) on the Illumina NextSeq 2000 sequencer. FASTQ files were aligned to the reference genome ASM265v1.54 with BWA 0.7.17-r1188. Single nucleotide variant (SNV) calling, indel calling, and annotation were accomplished with vcftools 1.9, Pindel 0.2.5b9, ANNOVAR v.$Date: 2020-06-07 23:56:37 -0400, and ASM265v1.54.gtf from Ensembl_r54 Afu. SNVs and indels were reviewed within *CYP51A, CYP51B, HMG1, HMG2,* and *HAPE*. **Results:** From 87 *A. fumigatus* isolates sequenced, 80 isolates passed sequencing quality metrics (5 = azole sensitive, 75 = azole resistant). *CYP51A* alterations were identified most frequently. The most common alterations were the previously described *CYP51A* promoter tandem repeats (21%, 16/75 including TR34/L98H, TR46/Y121F/T289A). *CYP51A* p.M220 alterations were identified in 9 azole-resistant isolates (12%), whereas *HAPE* p.P88L was identified in 1 azole-resistant isolate (1.3%). *HMG2* p.I235S was identified in 20% (15/75) of azole-resistant isolates, but also in 40% (2/5) of azole-susceptible isolates, supporting another recently described finding. Many other variants were identified in *CYP51A, HMG1*, and *HMG2* that were only found in azole-resistant isolates. **Conclusions:** We successfully performed whole-genome sequencing of 80 *A. fumigatus* isolates and characterized the prevalence of azole-resistance associated alterations. We found *CYP51A* alterations are most common in azole-resistant isolates. Although the *HMG2* p.I235S mutation has been reported as a possible mechanism of azole resistance in *A. fumigatus*, we confirm the variant is prevalent in azole-sensitive strains, showing this alteration is likely not responsible for azole resistance.

ID026. **Prospective Comparison of the BioFire Global Fever Panel to Laboratory-Developed PCR for Diagnosis of Suspected Malaria Patients**

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Introduction: Febrile illnesses in returning travelers can be associated with life-threatening tropical diseases including malaria, dengue fever, chikungunya, and leptospirosis. Due to their low prevalence in the United States, these illnesses may initially be missed due to their non-specific clinical presentation. However, these diseases may have high morbidity and mortality if left undiagnosed. To more quickly and accurately diagnose infection, we evaluated the analytical performance of the BioFire Global Fever Panel (GFP) compared to stand-alone laboratory-developed PCR tests (LDT) for malaria, dengue virus (DENV), and chikungunya virus (CHIKV). The GFP is simple for laboratories to adopt as it is FDA cleared, rapid, and requires very little hands-on time compared to LDTs. **Methods:** Sixty consecutive whole-blood specimens in ethylenediaminetetraacetic acid (EDTA) tubes were tested by LDT PCR for malaria. These specimens were then tested on the GFP within stability of the package insert. All samples tested for malaria were concurrently tested on the DENV and CHIKV LDT PCRs. Adjunct test orders from our laboratory for each of these patients were also reviewed in accordance with our IRB to analyze ordering practices. **Results:** The GFP detected at least 1 target in 14 of the 60 specimens (23.33% positivity). Of the 14 positives, 13 were positive for *Plasmodium spp.* by GFP and LDT. Four *P. falciparum* were identified by GFP and LDT. Nine specimens were detected as *P. vivax*/*P. ovale* by GFP. Seven of these were also identified as *P. vivax* or *P. ovale* by LDT and 2 were identified by LDT as *P. malariae.* Additionally, the GFP was able to accurately detect 1 DENV-positive patient, which was identified as DENV-2 by LDT. All negative specimens were negative by both GFP and LDT, for 100% negative agreement. Of the total 60 patients assessed, 10 patients also had DENV testing ordered (PCR and/or serology), and 2 patients had CHIKV testing ordered (PCR and/or serology). Due to the unavailability of a leptospirosis PCR at our institution, we were unable to test the *Leptospira* target on the GFP. **Conclusions:** The GFP accurately identified all positive patients but was unable to differentiate *P. malariae* from *P. vivax/ovale*, which is noted by the manufacturer for patients with high *P. malariae* parasitemia. As such, a positive *Plasmodium* spp. result should prompt further investigation to confirm species-level identification according to region of exposure. As the GFP had a 100% negative agreement with LDTs, this panel can be used to accurately rule out acute infection. As 16.7% of these patients were also undergoing work-up for suspected DENV or CHIKV infection, the GFP is a promising option to reduce cost, labor, and turnaround time when testing for multiple pathogens.