Methods. Retrospective analysis of 15,314 inpatients within the Mass General Brigham healthcare system who had two tests within a 36-hour period between May 1 2020 and May 29 2021. Early infection was defined as having a negative test followed by a positive test. Patients with prior positive tests were excluded. The primary outcome was the proportion of patients in early infection over the total number tested serially, stratified by 4-hour testing intervals from the timestamp of the first test. Multivariate modeling was used to identify features predictive of early infection. Covariates included demographics, body site, PCR assay, location, community incidence, percent positivity, and median / skew of Ct value distributions.

Results. Of 19,971 test pairs, 193 (0.97%) were characterized as a negative followed by a positive within 36 hours. Bivariate analysis showed a close association between negative to positive test pairs during the first surge in spring 2020 that was not present during the winter surge. Negative to positive test pairs were most common in the 12 to 16 hour time interval (51/193, 26%, Figure 1). After controlling for covariates, the Roche cobas assay was more likely to identify patients with a negative to positive test pair relative to the Cepheid Xpert, Hologic Panther Fusion and Roche Liat assays. A second specimen from the lower respiratory tract was more likely to lead to a positive relative to other body sites. Community incidence and Ct value distributions were not predictive and there were no differences between nasal and nasopharyngeal swabs. All 4-hour time intervals from 16 to 36 hours were significant for predicting a negative to positive test pair (Table 1).

Figure 1. Distribution of negative to positive test pairs by 4 hour time intervals

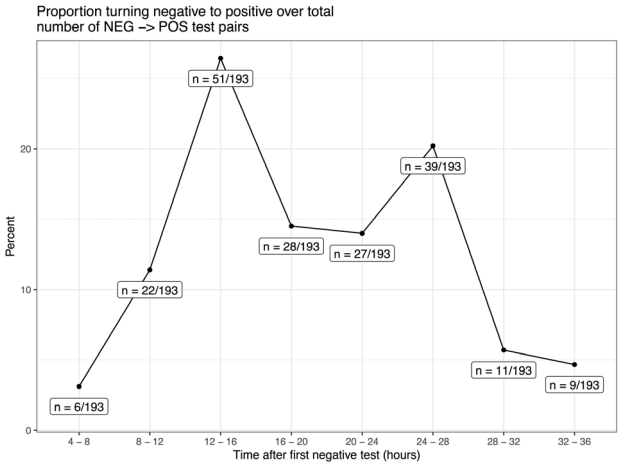

Table 1. Multivariate regression predicting a negative to positive test pair

Multivariate regression predicting a negative to positive test pair

Variable	Subgroup	OR	Lower 95% CI	Upper 95% CI	p value
Age		0.99	0.98	1.00	0.02
Gender (reference: Female)	Male	1.04	0.77	1.39	0.81
Month		1.00	1.00	1.00	0.08
Incidence in Boston		1.00	1.00	1.00	0.88
Percent positive across Mass General Brigham		1.10	0.97	1.25	0.15
Median Ct for Mass General Brigham		1.14	0.98	1.33	0.09
Skew of Ct distribution for Mass General Brigham		4.31	0.81	23.09	0.09
Assay for specimen 1 (reference: Cepheid Xpert)	Hologic Panther	1.33	0.88	2.01	0.17
	Roche cobas	1.92	1.13	3.24	0.01
	Roche Liat	0.84	0.15	3.52	0.83
Assay for specimen 2 (reference: Cepheid Xpert)	Hologic Panther	0.99	0.66	1.48	0.95
	Roche cobas	0.20	0.11	0.37	0.00
	Roche Liat	3.08	0.53	13.12	0.17
Body site for specimen 1 (reference: Nasopharynx)	Nasal	0.00	0.00	0.00	0.97
	Lower respiratory tract	0.67	0.16	1.94	0.52
	Other	3.73	0.21	18.45	0.20
Body site for specimen 2 (reference: Nasopharynx)	Nasal	1.78	0.29	6.00	0.43
	Lower respiratory tract	2.38	1.43	3.92	0.00
	Other	0.00	0.00	86067	0.98
Location category for specimen 1 (reference: ER)	Inpatient	1.19	0.80	1.76	0.39
Location category for specimen 2 (reference: ER)	Inpatient	0.86	0.55	1.38	0.52
Time interval between specimens (reference 4 - 8 hours)	8 - 12	1.22	0.51	3.37	0.68
	12 - 16	1.61	0.72	4.28	0.29
	16 - 20	2.71	1.16	7.40	0.03
	20 - 24	3.17	1.36	8.65	0.01
	24 - 28	4.76	2.10	12.85	0.00
	28 - 32	3.24	1.20	9.60	0.02
	32 - 36	3.72	1.31	11.31	0.01

Conclusion. The likelihood of detecting early infection is dependent on PCR platform and body site of sampling. A range of time intervals between 16 to 36 hours after the initial test were likely to identify positive cases.

Disclosures. **Sanjat Kanjilal, MD, MPH**, **GlaskoSmithKline** (Advisor or Review Panel member)

148. Single-amplicon, Multiplex Real-time RT-PCR with Tiled Probes to Detect SARS-CoV-2 *spike* Mutations Associated with Variants of Concern

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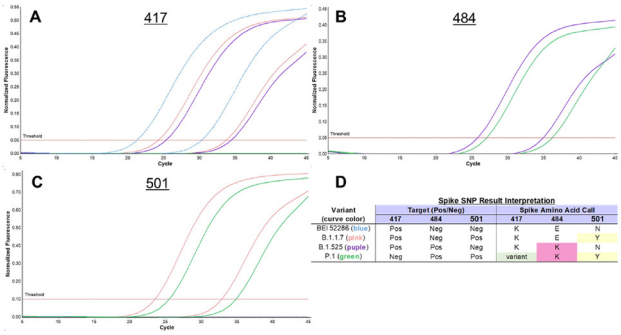
Session: O-30. Research in COVID-19 Diagnostics

Background. Detection and surveillance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants is of great public health importance. Broadly accessible and inexpensive assays are needed to enhance variant surveillance and detection globally. We developed and validated a single-reaction multiplex real-time RT-PCR (the Spike SNP assay) to detect specific mutations associated with variants of concern (VOC).

Methods. A single primer pair was designed to amplify a 348 bp region of *spike*. Probes were initially designed with locked nucleic acids (LNAs) to increase probe melting temperature, shorten probe length, and specifically detect 417K, E484K, and N501Y (**Figure**). The assay was optimized and evaluated using characterized variant sample pools. Clinical evaluation was performed on a convenience set of residual nasopharyngeal swabs, and variant calls were confirmed by SARS-CoV-2 genomic sequencing in a subset of samples. Following the initial evaluation, unmodified probes (without LNAs) were designed to detect L452R, L452Q, and E484Q.

Figure. Spike SNP distinguishes mutations occurring in different lineages (A-C).



Representative results of variant detection a single Spike SNP run are shown for mutations in the codons for 4177K (A) and mutations that encode 484K (B) and 501Y (C). Curves show dilutions of the following variants: blue, BEI 52286 (wild type); pink B.1.1.7; purple, B1.525; and green, P.1. Variant pools were used for B.1.17, B.1.525, and P.1 strains. Curves are displayed for a given dilution in each channel and result interpretation is shown (D).

Results. The lower limit of 95% detection was 2.46 to 2.48 $\log_{10}$ GE/mL for the three targets (~1-2 GE/reaction). Among 253 nasopharyngeal swabs with detectable SARS-CoV-2 RNA, the Spike SNP assay was positive in 238 (94.1%), including all samples with Ct values < 30 (220/220) for the N2 target and 18/33 samples with N2 Ct values ≥ 30. Results were confirmed by SARS-CoV-2 genomic sequencing in 50/50 samples (100%). Subsequent addition of the 452R probe did not affect performance for the original targets, and probes for 452Q and 484Q performed similarly to LNA-modified probes.

Conclusion. The Spike SNP assay provides fast, inexpensive and sensitive detection of specific mutations associated with SARS-CoV-2 VOCs, and the assay can be quickly modified to detect new mutations in the receptor binding domain. Similar analytical performance of LNA-modified and unmodified probes presents options for future assay customization that balance the shorter probe length (LNAs) and increased accessibility (unmodified). The Spike SNP assay, if implemented across laboratories offering SARS-CoV-2 testing, could greatly increase capacity for variant detection and surveillance globally.

Disclosures. **Colleen S. Kraft, MD, MSc**, Rebiotix (Individual(s) Involved: Self): Advisor or Review Panel member

149. Extraction-free RT-PCR to Detect SARS-CoV-2 Variants of Concern

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Background. SARS-CoV-2 variants of concern (VOC) have challenged real-time reverse transcriptase polymerase chain reaction (RT-PCR) methods for the diagnosis of COVID-19.

Methods. The CDC 2019-Novel Coronavirus real-time RT-PCR panel was modified to create a single-plex extraction-free proxy RT-PCR assay, VOCFast™. This assay uses the nucleocapsid N1 as well as novel primer/probe pairs to target VOC mutations in the Orf1a and spike (S) genes. For analytical validation of VOCFast, synthetic controls for the Wuhan, alpha/B.1.1.7, beta/B.1.351, and gamma/P.1 strains were tested at various concentrations. Clinical validation was performed using patient anterior nares swab and saliva specimens collected in the Denver, CO area between Nov 2020 and Feb 2021 or in March 2021. Orthogonal next-generation sequencing (NGS) was also performed.

Figure 1. Primer design for detection of SARS-CoV-2 variants of concern

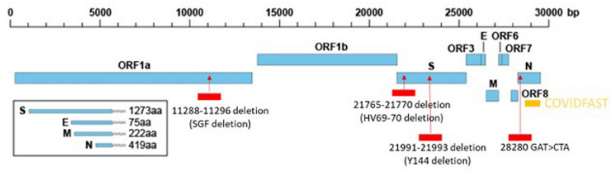
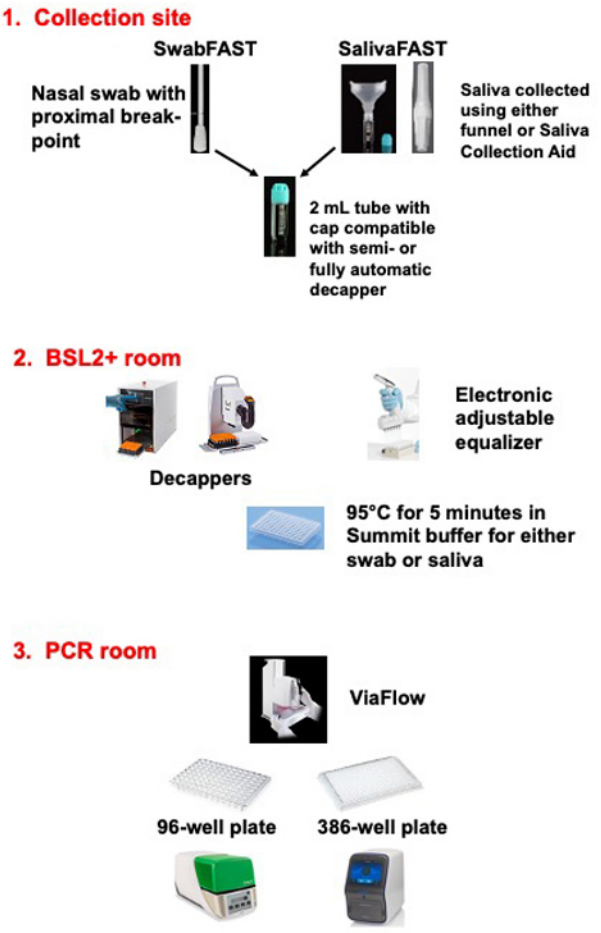


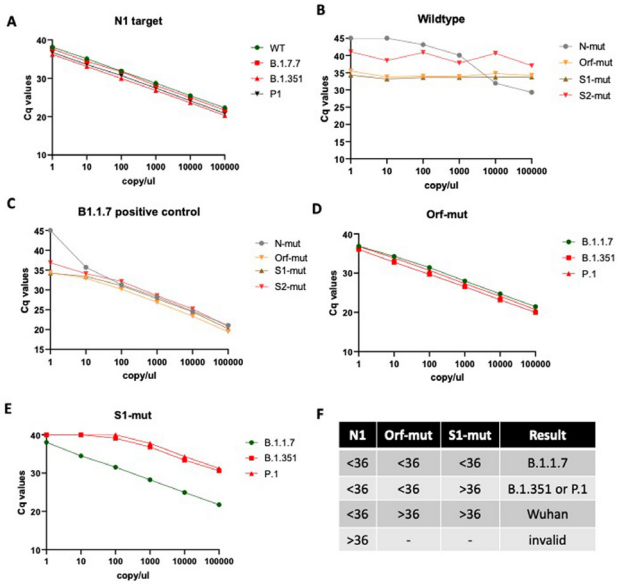
Figure 2. Process for testing by VOCFast



Results. Similar N1 quantification cycle (Cq) values corresponding to viral load were observed for all strains, suggesting that VOC mutations do not affect performance of the N1 primer/probe. Orf1a-mut and S1-mut primer/probes generated a stable high Cq value for the Wuhan strain. Conversely, Orf1a-mut Cq values were inversely correlated with viral load for all VOC. The S1-mut Cq was inversely correlated with viral load of the alpha strain, but did not reliably amplify beta/gamma VOC. The limit of detection was 8 copies/uL.

The first set of COVID-19 patient specimens revealed no amplification using Orf1a-mut whereas 53% of specimens collected in Mar 2021 demonstrated amplification by Orf-1a. Orthogonal testing by the SARS-CoV-2 NGS Assay and COVID-DX software demonstrated that 12/12 alpha strains, 2/2 beta/gamma strains, and 33/33 Wuhan strains were correctly identified by VOCFast.

Figure 3. Analytical validation of VOC primers/probes in wildtype (Wuhan) and VOCs



F	N1	Orf-mut	S1-mut	Result
	<36	<36	<36	B.1.1.7
	<36	<36	>36	B.1.351 or P.1
	<36	>36	>36	Wuhan
	>36	-	-	invalid

Figure 4. Limit of detection of VOCFast (Results)

	copies/uL	0	1	2	4	6	8	10	20
B.1.1.7	Orf-mut	NaN (0/3)	37.98 (0/3)	37.25 (0/3)	36.26 (1/3)	35.66 (2/3)	35.04 (3/3)	34.53 (3/3)	33.95 (3/3)
	S1	39.38 (0/3)	38.57 (0/3)	37.50 (0/3)	36.95 (0/3)	36.44 (0/3)	35.58 (3/3)	34.89 (3/3)	34.24 (3/3)
	N1	NaN (0/3)	38.25 (0/3)	36.62 (0/3)	35.47 (3/3)	34.87 (3/3)	34.55 (3/3)	34.73 (3/3)	34.01 (3/3)
B.1.351	Orf-mut	NaN (0/3)	37.39 (0/3)	36.86 (0/3)	35.23 (3/3)	34.53 (3/3)	34.18 (3/3)	33.76 (3/3)	32.75 (3/3)
	S1-mut	NaN (0/3)	NaN (0/3)	NaN (0/3)	38.28 (0/3)	39.50 (0/3)	NaN (0/3)	NaN (0/3)	NaN (0/3)
	N1	NaN (0/3)	38.16 (0/3)	35.79 (2/3)	34.48 (3/3)	34.04 (3/3)	33.53 (3/3)	33.10 (3/3)	32.24 (3/3)
P.1.	Orf-mut	NaN (0/3)	38.14 (0/3)	37.57 (0/3)	36.43 (1/3)	35.75 (2/3)	35.40 (3/3)	34.47 (3/3)	33.78 (3/3)
	S1-mut	39.56 (0/3)	NaN (0/3)	NaN (0/3)	NaN (0/3)	NaN (0/3)	NaN (0/3)	NaN (0/3)	NaN (0/3)
	N1	NaN (0/3)	36.83 (0/3)	36.13 (1/3)	34.67 (3/3)	34.48 (3/3)	34.03 (3/3)	33.49 (3/3)	32.83 (3/3)

Detection of VOC in clinical specimens and validation by NGS

Figure 5. Validation of VOCFast proxy PCR by NGS

A

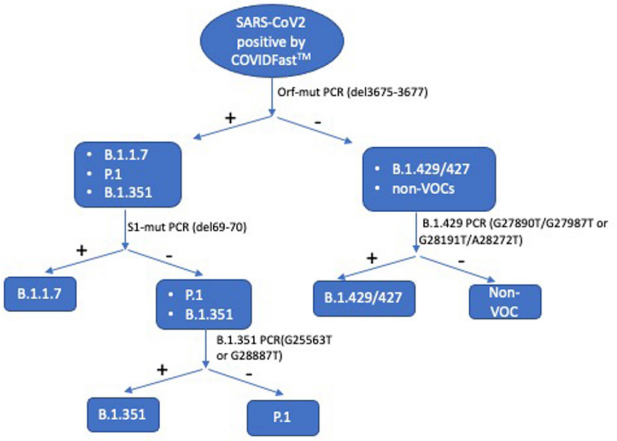
	Nov 2020 to Feb 2021	Mar 2021
Orf-mut positive	0/59 (0%)	17/32 (53.1%)

B

PCR-Orf-mut	PCR-S1-mut	NGS
Positive (12)	Positive (12)	B.1.1.7 (UK)---12/12
Positive (2)	Negative (2)	P.1. (Brazil)---1/2
		B.1.351 (South Africa)---1/2
Negative (33)	Negative (33)	B.1.429/427 (California)---4/33
		Non-VOCs---29/33

Conclusion. The combination of the N1, Orf1a-mut, and S1-mut primers/probes in VOCFast can distinguish the Wuhan, alpha, and beta/gamma strains and it consistent with NGS results. Testing of clinical samples revealed that VOC emerged in Denver, CO in March 2021. Future work to discriminate beta, gamma, and emerging VOC is ongoing. In summary, VOCFast is an extraction-free RT-PCR assay for nasal swab and saliva specimens that can identify VOC with a turnaround time suitable for clinical testing.

Figure 6. Schematic for VOC testing



Disclosures. **Brian L. Harry, MD PhD**, **Summit Biolabs Inc.** (Grant/Research Support, Shareholder) **Mara Couto-Rodriguez, MS**, **Biotia** (Employee) **Dorottya Nagy-Szakal, MD PhD**, **Biotia Inc** (Employee, Shareholder) **Niamh B. O'Hara, PhD**, **Biotia** (Board Member, Employee, Shareholder) **Shi-Long Lu, MD PhD**, **Summit Biolabs Inc.** (Grant/Research Support, Shareholder)

150. Updated Clinical Guidelines for Treatment and Prophylaxis of Plague

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Session: O-31. Respiratory Infections

Background. Plague still occurs naturally in the western United States, Latin America, Asia, and Africa. *Yersinia pestis*, the causative agent of plague, is a Tier 1 bioterrorism agent due to its potential for aerosol release and high fatality rates. Recommendations for treatment and post-exposure prophylaxis (PEP) of plague were published in 2000 and included limited first-line options for treating plague, namely streptomycin or gentamicin. Doxycycline or ciprofloxacin were recommended for PEP. However, since 2000 new human clinical data and animal data have become available, and the FDA has approved additional antimicrobials for plague.

Methods. CDC developed updated, evidence-based guidelines for treatment and prophylaxis of plague using a comprehensive process. To collect evidence on relative efficacy of various antimicrobials for treatment of plague, the guidelines team conducted systematic literature reviews and analyzed U.S. surveillance data. Results of these investigations were published in *Clinical Infectious Diseases* in 2020. We also hosted several meetings with subject matter experts and clinical organizations (IDSA, AAP, etc.), federal agencies, and others to review relevant data and gather individual input on treatment and prophylaxis of plague.

Results. The forthcoming plague guidelines will include several important updates. First-line treatment options have been expanded to include ciprofloxacin, levofloxacin, and moxifloxacin in addition to streptomycin and gentamicin. For PEP, levofloxacin and moxifloxacin are now first-line options in addition to doxycycline and ciprofloxacin. Trimethoprim-sulfamethoxazole is now one of several new alternative options for PEP. The updated guidelines also include recommendations for treatment of clinical forms of plague other than pneumonic. Additional special populations such as immunocompromised persons and neonates are also covered.

Conclusion. Plague remains a threat, both as a naturally occurring disease and as a potential bioterrorism weapon, and preparedness and early recognition are key to effective response. The updated clinical guidelines will be a useful tool for clinicians to manage antimicrobial treatment and PEP for plague.

Disclosures. **All Authors**: No reported disclosures

151. Simplifying Empiric Antimicrobial Therapy Selection for Lower Respiratory Tract Infections in Intensive Care Unit Patients: Using Resistance Frequency to Guide Decision Making

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Session: O-31. Respiratory Infections

Background. In the US, the burden of multidrug resistant bacterial infections, including carbapenem-resistant *P. aeruginosa* (CRPA) and ESBL-producing *Enterobacterales* (ESBL-E), is substantial. These resistant pathogens may affect the delivery of timely effective therapy. The aim of this study is to evaluate beta-lactam (BL) susceptibility trends based on the aggregate frequency of CRPA and a combined ESBL-E phenotype (*K. pneumoniae* (KPn) + *E. coli* (EC)) observed in critically ill patients with lower respiratory tract infections (LRTI).

Methods. In 2016-2019, ~20 US institutions per year submitted up to 250 gram-negative pathogens as part of the Study for Monitoring Antimicrobial Resistance Trends. A total of 871 PA, 380 KPn, and 336 EC isolates were collected from ICU patients with LRTI. MICs were determined using broth microdilution and interpreted using 2021 CLSI breakpoints. ESBL-E phenotype was defined as: ceftriaxone MIC ≥ 2 mcg/mL. Institutions were stratified into two groups based on frequency of CRPA and combined ESBL-E phenotype: Group 1: CRPA ≤ 15% and ESBL-E ≤ 15%; Group 2: CRPA > 15% and ESBL-E > 15%. Based on CLSI guidance, an empiric antibiotic susceptibility threshold of ≥90% was deemed optimal.

Results. Overall, CRPA and ESBL-E phenotypes were identified in 28.4% and 21.2% of isolates, respectively. Aggregate BL susceptibility in group 1 was above the 90% threshold for cefepime (FEP), piperacillin/tazobactam (TZP), meropenem (MEM), ceftolozane/tazobactam (C/T), and imipenem/relebactam (I/R) (Table 1). However, as frequency of CRPA and ESBL-E exceeded 15%, aggregate BL susceptibility declined to 77.3%, 79.3%, and 86.2% for FEP, TZP, and MEM, respectively. In contrast, C/T and I/R maintain susceptibility above the empiric susceptibility threshold.

Table 1. Aggregate susceptibility of P. aeruginosa, E. coli, and K. pneumoniae ICU LRTI isolates stratified by resistance frequency: Best- (Group 1) and worst-case (Group 2) scenarios

Organism	n	C/T	I/R	FEP*	TZP	MEM
Group 1: CR *P. aeruginosa* and Combined ESBL-E Phenotype ≤ 15% of isolates						
P. aeruginosa	203	97.5	98.5	88.2	85.3	94.1
E. coli	123	97.6	100	98.4	92.7	100
K. pneumoniae	127	97.6	99.2	96.9	94.5	99.2
Aggregate Susc		**97.6**	**99.2**	**94.5**	**90.8**	**97.8**
Group 2: CR *P. aeruginosa* and Combined ESBL-E Phenotype > 15% of isolates						
P. aeruginosa	668	92.8	88.5	70.1	62.9	64.8
E. coli	213	94.8	100	78.4	89.2	98.1
K. pneumoniae	253	91.3	99.2	83.3	85.8	95.6
Aggregate Susc		**93.0**	**95.9**	**77.3**	**79.3**	**86.2**

C/T: Ceftolozane/tazobactam; I/R: Imipenem/relebactam; FEP: Cefepime; TZP: Piperacillin/tazobactam; MEM: Meropenem; Susc: Susceptibility
*Cefepime susceptibility includes Susceptible Dose-Dependent isolates
Green Highlight: At or above CLSI's empiric therapy susceptibility threshold of ≥ 90%.
Red Highlight: Below CLSI's empiric therapy susceptibility threshold of ≥ 90%.

Group 1 institutions: *P. aeruginosa* (N = 32); Combined ESBL-E Phenotype (N = 30)
Group 2 institutions: *P. aeruginosa* (N = 48); Combined ESBL-E Phenotype (N = 50)

Conclusion. In ICU patients, exceeding CRPA and combined ESBL-E phenotype frequency of 15% for both classifications, impacts susceptibility to 1st line BL's resulting in a failure to achieve empiric susceptibility thresholds. This stratification could serve as a decision point for triggering earlier susceptibility testing or modifying empiric therapy recommendations for LRTI to include newer agents pending microbiology results.

Disclosures. **Kenneth Klinker, PharmD**, **Merck & Co., Inc.** (Employee, Shareholder) **Levita K. Hidayat, PharmD BCIDP**, **Merck & Co., Inc.** (Employee, Shareholder) **C. Andrew DeRyke, PharmD**, **Merck & Co., Inc.** (Employee, Shareholder) **Mary Motyl, PhD**, **Merck & Co., Inc.** (Employee, Shareholder) **Karri A. Bauer, PharmD**, **Merck & Co., Inc.** (Employee, Shareholder)

152. Sharp Decline in Rates of Community Respiratory Viral Infections Among NIH Clinical Center Patients During the COVID-19 Pandemic

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Session: O-31. Respiratory Infections

Background. During the first year of the COVID-19 pandemic, nonpharmaceutical interventions had a broad impact on viral transmission apart from SARS-CoV-2. The NIH Clinical Center has used the BioFire FilmArray multiplex PCR respiratory pathogen panel (RPP) for evaluation of upper respiratory symptoms since 2014. Beginning in 3/20, respiratory samples from symptomatic patients were tested by SARS-CoV-2 PCR and the RPP. We performed a retrospective study comparing frequency and rates of community respiratory viruses detected by RPP from 1/14 through 3/21.

Methods. Results of RPPs from nasopharyngeal swabs/washes, bronchoalveolar lavages, and bronchial washes were included. Results from viral challenge studies were excluded. Charts were reviewed to determine whether repeat positives for the same virus within 12 months represented new infections; repeats from the same infection were excluded. A quantitative data analysis was completed using cross tabulations; comparisons were done using mixed models, applying Dunnett's correction for multiplicity.

Results. A total of 3,329 patients underwent 8,122 RPPs from 1/14 through 3/21. Frequency of all respiratory pathogens declined from an annual range of 0.88-1.97% from 1/14-3/20 to 0.29% in 4/20-3/21 ($p < 0.001$). Individual viral pathogens declined sharply in frequency during the pandemic, with zero cases of influenza A/B, parainfluenza, or metapneumovirus detected from 4/20-3/21. One case each of adenovirus, RSV, CoV OC43, and CoV HKU1 were detected in 4/20-3/21. Rhino/enterovirus detection