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Clinical Research Protocol

Healthcare-associated links in transmission of nontuberculous mycobacteria among patients with cystic fibrosis

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Jerry A. Nick, MD *Date*

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PROTOCOL AGREEMENT

I have read the protocol specified below. In my formal capacity as Investigator, my duties include ensuring the safety of the study participants enrolled under my supervision and providing the Sponsor-Investigators with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site. Furthermore, on behalf of the study staff and myself, I agree to maintain the procedures required to carry out the study in accordance with accepted GCP principles and to abide by the terms of this protocol.

Protocol Number: HALT NTM

Protocol Title: Healthcare-associated links in transmission of nontuberculous mycobacteria among patients with cystic fibrosis (HALT NTM)

Protocol Date: May 8, 2020

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Investigator Signature *Date*

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Site #: _____

Site Name: _____

Address: _____

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LIST OF ABBREVIATIONS AND ACRONYMS

AE	Adverse event
CFR	Code of Federal Regulations
CFTR	Cystic fibrosis transmembrane conductance regulator
CF	Cystic fibrosis
CFF	Cystic Fibrosis Foundation
CFF PR	CFF Patient Registry
CO-RDP	Colorado Research Development Program
DMC	Data Monitoring Committee
DSMB	Data Safety Monitoring Board
EDC	Electronic data capture
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act of 1996
IP&C	Infection prevention and control
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IRB	Institutional Review Board
MAC	*M. avium* complex
NTM	Nontuberculous mycobacteria
PI	Principal Investigator
REDCap	Research Electronic Data Capture
TDNCC	Therapeutics Development Network Coordinating Center
WGS	Whole genome sequencing

PROTOCOL SYNOPSIS

TITLE	Healthcare-associated links in transmission of nontuberculous mycobacteria among patients with cystic fibrosis (HALT NTM)
SPONSOR-INVESTIGATOR	Jane E. Gross, MD PhD Jerry A. Nick, MD
FUNDING ORGANIZATION	Cystic Fibrosis Foundation (CFF)
NUMBER OF SITES	Up to 20
RATIONALE	Pulmonary NTM infection is recognized as one of the most challenging infections to treat among CF patients, notable for prolonged treatment courses and often poor response to therapy. Positive cultures for NTM occur in about 20% of children and adults with CF. However, the source of NTM infection, modes of transmission, and exposure risks are poorly understood. It is thought that NTM is primarily acquired from environmental sites including soil and water as well as water supply systems to homes, hospitals, and clinics and from aerosols generated by flowing water from taps, showers, and fountains. Nonetheless, no direct molecular link has been established between environmental NTM and respiratory CF NTM. Healthcare-associated transmission of NTM among CF patients has been suspected and is of growing concern for CF Centers worldwide. Widespread global transmission of NTM, potentially via person-to-person transmission of fomites and aerosols has been reported, but no standardized epidemiologic investigation tool for healthcare-associated NTM acquisition has been published.
STUDY DESIGN	**PART A / Epidemiologic Investigation:** The CO-RDP provides a national reference laboratory for CF NTM. NTM respiratory isolates received from CF Care Centers around the U.S. undergo culture, molecular identification, antimicrobial susceptibility, and whole genome sequencing (WGS). Using this approach, the CO-RDP has identified clusters of NTM isolates, defined as highly similar strains at the genomic level, harbored by two or more CF patients who are cared for at the same CF Care Center. These identifications have heightened our concern for potential healthcare-associated NTM acquisition originating from patient-to-patient transmission or a common environmental source within Centers. Using integrated clinical and epidemiological research methods, the HALT NTM retrospective epidemiologic investigation can identify overlaps in source(s) of care between patients with highly similar NTM isolates in a Center. The HALT NTM toolkit facilitates a stepwise process by which individual Centers perform retrospective epidemiologic evaluation of patients identified by the CO-RDP as part of an NTM cluster. HALT NTM is available to the entire CF

	Foundation Care Network, under a collaborative agreement, to initiate a standardized, independent, confidential, internal NTM outbreak investigation. A control comparison group will also be investigated. **PART B / Water Biofilm Collection**: Clustered NTM isolates could originate from a shared healthcare water source. Biofilms from healthcare water supplies will be collected and NTM recovered, identified, and sequenced to determine if the respiratory CF NTM strain genotype is similar to those recovered from the healthcare water supply.
PARTICIPANT STUDY DURATION	**PART A/ Epidemiologic Investigation:** CF Centers will be eligible for enrollment as NTM clusters are identified by the CO-RDP. The total duration of the study and analysis is 3 years. **PART B / Water Biofilm Collection:** CF Centers will be eligible for enrollment as NTM clusters are identified by the CO-RDP. The total duration of the study and analysis is 3 years.
PRIMARY OBJECTIVE	**PART A / Epidemiologic Investigation:** Our primary objective is to implement a standardized epidemiologic investigation, via the HALT NTM tool kit, in CF Care Centers identified as having clusters of highly similar NTM isolates. We will assess the origins and prevalence of healthcare associated patient to patient transmission of NTM among different CF Care Centers. We will characterize the source(s) of direct or indirect patient-to-patient transmission of NTM within an individual CF healthcare setting. **PART B / Water Biofilm Collection:** The primary objective is to determine if NTM strains identified in clusters are related to strains isolated from water biofilm sources in the CF healthcare setting. Because hospital and clinic plumbing sources could pose a risk to CF patients, we aim to perform WGS of NTM isolates from healthcare biofilms obtained from CF Centers with NTM clusters among patients to determine if the biofilm isolates are highly related to the isolates recovered from patients.
SECONDARY OBJECTIVES	**PART A / Epidemiologic Investigation:** The secondary objectives are to: ▪ Assess degree of proximity of subjects in time and/or space. ▪ Compare the relationship between similarity of strains and degree of proximity of subjects. ▪ Model the relationship between the degree of similarity and strains with the proximity and subject characteristic variables. **PART B / Water Biofilm Collection:**

	The secondary objectives are to: ▪ Characterize clinically-relevant NTM isolates and healthcare biofilms. ▪ Describe most common biofilm sources within the healthcare setting harboring clinically relevant NTM isolates.
NUMBER OF PARTICIPANTS	**PART A / Epidemiologic Investigation:** Unlimited and based on the number of patients identified in an NTM cluster within an individual CF Care Center (prospectively collected data). Two controls will be identified for every subject within a cluster. **PART B / Water Biofilm Collection:** N/A
PARTICIPANT SELECTION CRITERIA: **Inclusion Criteria**	**PART A / Epidemiologic Investigation:** Inclusion Criteria: 1. Written informed consent is not required for this retrospective epidemiological study. 2. Diagnosis of CF consistent with the 2017 CFF guidelines. 3. Male or female participant of any age who have a history of NTM **species or sub-species** collected from expectorated sputum, induced sputum and/or bronchoalveolar lavage and identified by the CO-RDP as falling into a highly-related cluster within a single CF Care Center. 4. Controls subjects are male or female participants of any age who have a history of NTM **species or sub-species** collected from expectorated sputum, induced sputum and/or bronchoalveolar lavage and identified by the CO-RDP as NOT falling into a highly-related cluster within a single CF Care Center. **PART B / Water Biofilm Collection:** Inclusion Criteria: N/A
PARTICIPANT SELECTION CRITERIA: **Exclusion Criteria**	**PART A / Epidemiologic Investigation:** N/A **PART B / Water Biofilm Collection:** N/A
CONCOMMITANT MEDICATIONS	**Allowed:** N/A **Prohibited:** N/A
PRIMARY ENDPOINT	**PART A / Epidemiologic Investigation:** Identification of a shared healthcare-associated source(s) between patients in a CF Care Center. **PART B / Water Biofilm Collection:**

	Identification of biofilm isolates that are highly related to the isolates recovered from patients.
SECONDARY ENDPOINTS	**PART A / Epidemiologic Investigation:** The secondary endpoints are: ▪ Incidence and prevalence of CF NTM species/subspecies by geographical region. **PART B/ Water Biofilm Collection:** The secondary endpoints are: ▪ Incidence and prevalence of healthcare-associated water biofilm NTM species/subspecies by geographical region.
EXPLORATORY EVALUATIONS	None
PLANNED INTERIM ANALYSIS	No formal interim analysis involving a data monitoring committee (DMC) is planned.
STATISTICS PRIMARY ANALYSIS PLAN	**PART A / Epidemiologic Investigation:** For the primary endpoint, biostatistical analysis of the multicenter data will be completed using both qualitative and quantitative approaches. We will identify a set of variables used to assess degree of proximity of subjects in time and/or space. For example, two subjects with neighboring rooms at a hospital would have close proximity, but proximity would be increased for two subjects at a facility that had longer overlap time, relative to a pair of subjects that had shorter overlap. We will explore creating composite measures of quantitative proximity that incorporate multiple variables. Qualitative measures of proximity, such as location type (testing laboratory, patient room) will also be assessed. Several testing approaches will be used to compare the relationship between similarity of strains and degree of proximity of subjects. In the simplest form, descriptive statistics will be computed for proximity variables, for pairs of subjects who are determined to have similar strains versus those who have dissimilar strains, and multivariate or 2-sample t-tests will be performed to test for differences between these groups. More advanced modeling will be performed to determine the relationship between the degree of similarity in strains with the proximity and subject characteristic variables; multiple linear regression will be used when considering continuous strain similarity, while multiple logistic regression will be used when considering subjects with dichotomized (similar/dissimilar) strains to other subjects.

	PART B / Water Biofilm Collection: Several testing approaches will be used to compare the relationship between similarity of strains between the clustered patients and environmental isolates. Descriptive statistics will be computed for proximity variables, for pairs of subjects and environmental isolates that are determined to have similar strains versus those who have dissimilar strains, and multivariate or 2-sample t-tests will be performed to test for differences between these groups.
RATIONALE FOR NUMBER OF PARTICIPANTS	The CO-RDP provides a national reference laboratory for CF NTM. NTM respiratory isolates received from CF Care Centers around the U.S. undergo culture, molecular identification, antimicrobial susceptibility, and WGS. Using this approach, the CO-RDP has identified clusters of NTM isolates, defined as highly similar strains at the genomic level, harbored by two or more CF patients who are cared for at the same CF Care Center. All CF Centers, found to have clusters of highly-related NTM isolates will be offered enrollment. Subject enrollment is unlimited and based on the number of patients identified in an NTM cluster within an individual CF Care Center (prospectively collected data).

1 BACKGROUND

Healthcare-associated transmission of NTM

Pulmonary nontuberculous mycobacteria (NTM) infection is recognized as one of the most challenging infections to treat among cystic fibrosis (CF) patients, notable for prolonged treatment courses and often poor response to therapy.[1,2] Positive cultures for NTM occur in about 20% of children and adults with CF.[3] However, the source of NTM infection, modes of transmission, and exposure risks are poorly understood. It is thought that NTM is primarily acquired from environmental sites including soil and water as well as water supply systems to homes, hospitals, and clinics and from aerosols generated by flowing water from taps, showers, and fountains.[4-6] Nonetheless, no direct molecular link has been established between environmental NTM and respiratory CF NTM.[7] Healthcare-associated transmission of NTM among CF patients has been suspected[8-10] and is of growing concern for CF Centers worldwide. Bryant *et al.* reported widespread global transmission of NTM, potentially via person-to-person transmission of fomites and aerosols,[11] but no standardized epidemiologic investigation tool for healthcare-associated NTM acquisition has been published.

CO-RDP serves as a national reference laboratory for CF NTM

The Colorado Research Development Program (CO-RDP) provides a national reference laboratory for CF NTM.[12] NTM respiratory isolates received from CF Care Centers around the U.S. undergo culture, molecular identification, antimicrobial susceptibility, and whole genome sequencing (WGS).[13] Using this approach, the CO-RDP has identified clusters of NTM isolates, defined as highly similar strains at the genomic level, harbored by two or more CF patients who are cared for at the same CF Care Center. These identifications have heightened our concern for potential healthcare-associated NTM acquisition originating from patient-to-patient transmission or a common environmental source within Centers.

Identification of highly similar clusters of NTM within the care center network

Analysis of the 2016 CF Foundation (CFF) Patient Registry Annual Data Report found 20% of patients cultured for NTM between 2012-2016 had a NTM species isolated at least once.[17] In 2017, of the 15,041 CF patients in the national registry who had an NTM culture performed that year, 1,903 (12.7%) had an NTM species isolated one or more times.[18] The modes of acquisition of NTM in CF remain unclear (**Figure 1**).

The CO-RDP comprises multiple coordinated cores which receive, bank, culture, sequence, and analyze NTM clinical isolates from CF clinics around the U.S. Extracted NTM DNA from received samples is sequenced via WGS and analyzed for phylogenetic relatedness. As of January 2019, 123 facilities across the nation have submitted CF NTM isolates that have been sequenced. Phylogenetic data of NTM isolates from CF patients in the U.S. (**Figure 2**), as well as reference and environmental stains worldwide, are being collected in an ongoing fashion by the CO-RDP, and we found that highly similar NTM strains are shared within individual Care Centers (**Figure 3**) as well as between geographically

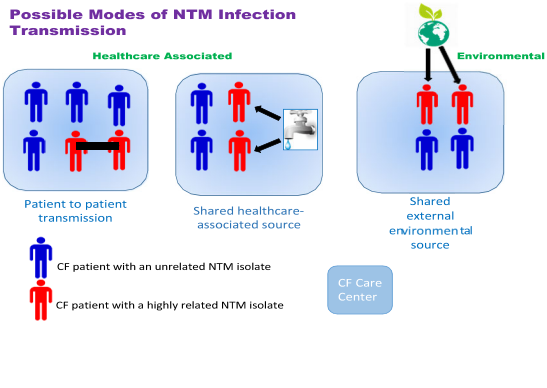

Figure 1: Possible modes of NTM transmission.

distant Centers. This work is clinically significant because a shared highly similar strain within a healthcare setting suggests common environmental sources of acquisition or patient- to-patient transmission.[9]

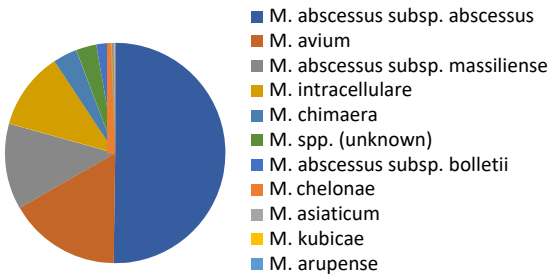

Figure 2: CF NTM isolates sequenced by the CO-RDP.

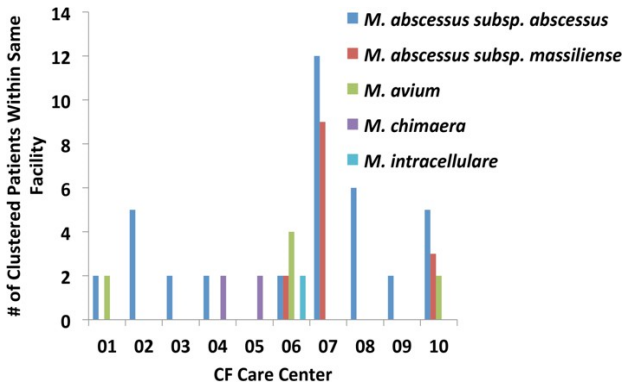

Figure 3: CO-RDP identified NTM clusters within 10 CF Care Centers.

To define relatedness, the CO-RDP has examined the range of single-nucleotide polymorphism (SNP) differences among isolates from different patients compared to longitudinal isolates from the same patients over time to establish an evidence-based SNP threshold that is consistent with a clonal or highly similar strain within each NTM species.[13,19] As this genomic database expands, thresholds of similarity will be refined through continued acquisition of longitudinal isolates collected from patients. Together, the inter- and intra-patient information defines a threshold to initiate an epidemiologic investigation. As the number of SNPs between strains increases, the likelihood of shared origin decreases. SNP threshold numbers are also dependent on the size of the core genome used to assess similarity. The CO-RDP utilizes WGS and phylogenomic analysis to examine the population structure and diversity of each NTM species and subspecies, and to identify clustered strains that are phylogenetically-related and nearly identical at the core genome level. Using the CO-RDP data driven methodology, a threshold of 15 SNP for *M. abscessus* ssp. *abscessus* (MAB) is stringent, reliable and provides an optimal approach to assess similarity between isolates.[13,19]

Multiple reports concerning healthcare associated outbreaks of NTM among CF patients continue to appear in the literature.[8-10] Despite the reports, no standardized approach to epidemiologic investigation has been established for examination of healthcare-associated CF NTM outbreaks. Criteria must be met to validate NTM disease transmission.[20] NTM bacterial isolates must be genetically-matched between the patient and environment or between patients. Evidence of epidemiologic exposure and potential cross-infection must exist. Currently, ***WGS is the gold standard for NTM isolate comparison*** and was used in the study at Papworth Hospital to assess transmission of NTM between CF patients.[9] The authors examined WGS on 168 consecutive isolates of *M. abscessus* from 31 adult CF patients in a Center from 2007-2011. Using core genome SNP analysis, they identified highly similar (<25 SNP) clusters of *M. abscessus massiliense* (MMAS) isolates from patients who demonstrated opportunities for cross- infection via overlapping clinic visits using epidemiological data and contact investigation. The authors concluded substantial evidence existed to support ***healthcare-acquired transmission***. One smaller study utilized a similar approach to evaluate highly similar NTM isolates, but the data did not support healthcare-associated transmission of infection.[21]

1.1 Overview of Non-Clinical Studies

Not Applicable

1.2 Overview of Preliminary Studies

When the CO-RDP receives NTM clinical isolates from around the U.S., DNA is sequenced via WGS and analyzed for genetic relatedness at the core genome level. Mycobacterial DNA is sequenced using Illumina MiSeq 2X300 chemistry. Sequence reads are mapped to the appropriate mycobacterial reference genome and compared to find phylogenomic differences at the SNP level. As of January 2019, over 1,000 CF NTM isolates from 631 patients at 92 contributing facilities have ***been banked***. To date, 904 CF NTM isolates ***have been sequenced from 79 U.S. Care Centers, representing a total of 13 predominant NTM species and subspecies*** (**Figure 2**). To determine a threshold of genetic similarity for highly similar isolates, pairwise SNP distances in the core genome were calculated between all pairs of "same" (within- patient) or "different" (between-patient) isolates. We observed that 25 or fewer SNPs define background similarity of same-patient isolates for MAB and MMAS. Thus, a conservative threshold of 15 SNPs was chosen to define genetic clusters between patients, representing likely transmission events or shared source acquisition.

Findings: Unpublished data from the CO-RDP of 114 isolates from CF MAB patients across the country demonstrates 26 of 114 (23%) had isolates in highly related clusters. ***Twelve of 114 (11%) had isolates in clusters and received care in the same Center. Clusters are occurring*** among multiple ***M. abscessus*** **subspecies as well as** ***Mycobacterium avium*** **complex (MAC) subspecies**.

Preliminary results from one Care Center utilizing the HALT NTM toolkit investigated six clusters of patients with highly similar respiratory NTM isolates, including *M. abscessus* and MAC. The size of the highly related clusters ranged from 2-3 patients. Among the six identified NTM clusters, two clusters occurred with MAB, one with MMAS*, two with M. avium* (MAV)*, and one with M. intracellulare.* No patients within a cluster are related or living together. This is the first description of a MAC cluster.

Cluster 1 consisted of 3 subjects with highly related MAV. **Subject A** was defined as the patient with the longest period with MAV+ culture. **Subject B** was defined as the patient with the second longest period with MAV+ culture. **Subject C** was defined as the patient with the shortest period with MAV+ culture. Subjects in Cluster 1 were compared using the following pair comparison: AB, AC, and BC. The abstraction timeframe for Pair AB was 67 months, Pair BC was 36 months, and Pair AC was 79 months. Results of the HALT NTM toolkit revealed Subjects A and B had a 1-day CF clinic overlap (**Figure 5**). At that time, Subject A cultured positive for MAV, but Subject B did not. Local clinic IP&C standards at that time required patients to don a mask when outside of a clinic room and required gown and gloving by physicians, but not ancillary staff. Subjects A and B were seen by different physicians and different PFT technicians. Points of overlap during the clinic visit included: Subjects A and B had a clinic visit on the same afternoon, waited in a shared waiting room, underwent spirometry in a common access PFT laboratory room, and had a nutrition consult within the subject's clinic room with the same nutritionist. Subject B cultured positive for MAV 25 months after the Subject AB overlap. Subjects B and C had a 1-day overlap in the clinic 43 days after Subject B first cultured positive for MAV. Points of overlap included: Subjects B and C were seen by the same nutritionist on the same afternoon in CF clinic as well as waiting in a shared waiting room, and spirometry in a common access PFT laboratory room prior to being placed in their respective clinic room. Subject C first cultured positive for MAV 12 months after the Subject BC overlap. There was no overlap among any of the Cluster 1 subjects in the hospital setting.

Cluster 2 consisted of 2 subjects with highly related MAB. **Subject A** was defined as the patient with the longest period with MAB+ culture. **Subject B** had the shortest period with MAB+ culture. The abstraction timeframe for Pair AB was 81 months. Results of the HALT NTM toolkit revealed Subjects A and B had a 15-day hospitalization overlap. Subject A first cultured positive for MAB 9 days into the 22-day admission when overlap occurred. Subject B

cultured positive for MAB 14 days into the 16-day admission and 4 days after Subject A. Local hospital IP&C standards at that time required patients to don a mask when outside of a clinic room and required gown and gloving by all staff entering the subject's room. Subjects A and B were physically separated by one hospital room. At that time, CF patients had privileges to walk unaccompanied through the hospital while donning a mask and had access to a common coffeemaker (Keurig) and sink.

Cluster 3 consisted of 2 subjects with highly related MAB. Abstraction timeframe was 42 months. **Cluster 4** consisted of 2 subjects with highly related MMAS. Abstraction was 28 months. **Cluster 5** consisted of 2 subjects with highly related *M. intracellulare.* Abstraction was 29 months. Subjects were paired as previously defined. Results of the HALT NTM toolkit revealed there was no overlap identified among subjects in Clusters3, 4, or 5 in the CF clinic or hospital setting.

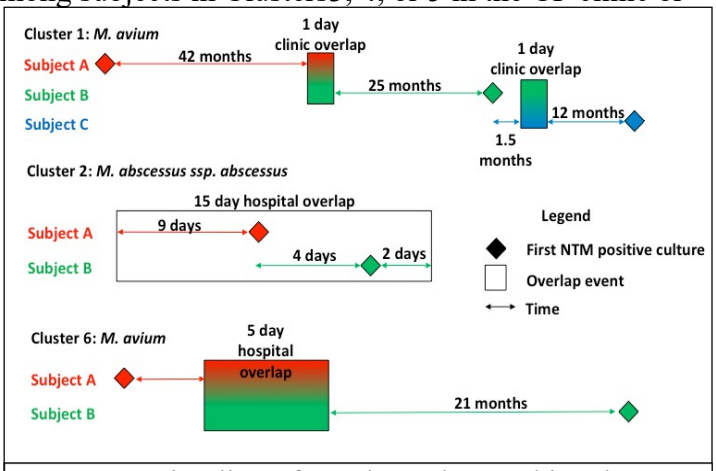

Figure 5: Timeline of overlaps observed in Clusters 1, 2, and 6.

Cluster 6 consisted of 2 subjects with highly related MAV. Subjects were paired as previously defined. The abstraction timeframe for Pair AB was 45 months. The HALT NTM toolkit revealed Subjects A and B had one 5- day hospitalization overlap. Subject A was culture positive for MAV prior to the first day of the admission when overlap occurred. Points of overlap included: Subjects A and B both had spirometry and a physical therapy consult on the same day during the admission overlap. Local hospital IP&C standards at that time required patients to don a mask when outside of a clinic room and required gown and gloving by all staff entering the subject's room. CF patients had privileges to walk unaccompanied through the hospital while donning a mask and had access to a common coffeemaker (Keurig) and sink. Subject B cultured positive for MAV 21 months after the Subject AB hospitalization overlap. There was no overlap among the Cluster 6 subjects in the clinic setting. The timeline of overlap for Clusters 1, 2, and 6 are shown (**Figure 5**).

Four sets of paired control patients with MAB were also compared utilizing the HALT NTM toolkit. Control patients were identified as having isolates that were determined to be unique and unrelated. In all 4 control patient pairs with unrelated strains no healthcare-associated points of overlap were identified. The abstraction timeframe for the control pairs ranged from 4-7 years.

Discussion: Martiniano *et. al*. evaluated the clinical significance of a first positive CF NTM culture and reported subjects that develop active NTM disease have a lower baseline forced expiratory volume in 1 second (FEV_1) at the time of first positive culture and an increased rate of decline in FEV_1 one year preceding the first positive culture.[36] ***The finding of accelerated lung function decline in patients with NTM disease before the time of first positive culture strongly suggests the presence of respiratory NTM in CF patients for a year or more before initial clinical detection.*** Based on that knowledge, the timeline for biologically plausible acquisition prior to detection is reasonably estimated to be 1-2 years. For this reason, the abstraction timeframe in the HALT NTM toolkit timeframe begins 2 years prior to the first positive culture of the patient with the longest period of time with NTM+ culture. The timeframe ends when the second paired patient has a first positive culture. This approach to setting the timeline for data

abstraction ensures plausible directionality of transmission and allows for a 1-2 year timespan from NTM acquisition to NTM culture positivity.

Cluster 1 suggests the possibility of indirect patient-to-patient transmission of MAV from **Subject A** to **Subject B**, with **Subject B** becoming culture positive for the same highly related strain 25 months later. Forty-three days after **Subject B** became culture positive, there was a clinic overlap with Subject C. Twelve months after the BC clinic overlap, **Subject C** became culture positive for the same highly related MAV as Subjects A and B. In this cluster, we observed a possible instance of indirect patient-to-patient contact involving the PFT laboratory and/or a single nutritionist, not donning gown and gloves, in both Pairs AB as well as BC. Based on the most recent CF IP&C guidelines, a plausible bacterial transmission event may occur with horizontal surface contamination of equipment such as in the PFT laboratory and/or involve surface contamination of staff skin and clothing when not donning protective, removable gowns and gloves while caring for CF patients.[26] There is no evidence to support healthy carrier status as a source of NTM transmission. We hypothesize that transmission may have occurred via droplets ***carried on the exposed skin or clothing of staff caring for multiple CF patients or by common equipment that is used among multiple CF patients***. In **Cluster 2**, both subjects became MAB+ within 4 days of each other during a 15-day hospitalization overlap. Based on the rapid succession of newly positive respiratory MAB cultures among two patients during hospitalization, this cluster appears to be ***a possible candidate for environmental healthcare- associated infection***. Both subjects were exposed to the same environment and resided within a one room distance from each other. Determining if the clinical NTM strains are related to the strains isolated from water biofilm sources in the hospital would more definitively link the origin of NTM acquisition within the healthcare setting. ***This cluster demonstrates the need for simultaneous environmental sampling of the healthcare environment as planned in Aim 2.*** **Cluster 6** suggests possible indirect patient-to-patient transmission of MAV via droplets carried on common equipment or shared patient resources such as the coffee maker or sink. With standard precautions of gown and glove requirement for all staff entering a patient room during hospitalization, indirect transmission of droplets carried by contaminated skin or clothing of staff is less likely since all protective barriers are removed prior to leaving a patient room. Common acquisition of a highly related strain from the environment also seems unlikely given Subject A was MAV culture positive prior to hospital admission. **Clusters 3-5** did not identify any healthcare-associated subject overlap.

In this study, we are unable to exclude NTM acquisition from direct patient-to-patient contact or via a common environmental source outside the healthcare setting. Evaluation of non-healthcare settings is beyond the scope of this project, but serves as a potential future area of investigation. There may also be a baseline frequency of patient overlap that is not related to transmission events in the healthcare setting that we expect to capture with evaluation of control pairs of subjects. Despite this small sample size, this preliminary data provides insight into the frequency and nature of patient overlap in the healthcare setting and reveals that among CF patients receiving healthcare in the same location, there are opportunities for close direct or indirect contact. In the setting of strict IP&C measures with single patient rooms, healthcare staff gowning and gloving, patients wearing masks, aggressive cleaning procedures between patients, and maintenance of patient-to-patient separation of 6 feet or more, direct and indirect patient overlap should be minimized. However, incidental or unanticipated contacts remain a risk. If patients in fact have no direct or indirect contact, ***investigation of the healthcare environment is essential to eliminate a common healthcare-associated environmental source as the point of CF patient NTM acquisition.***

2 STUDY RATIONALE

Reports concerning for direct or indirect healthcare-associated NTM transmission continue to appear in the literature.[8-10] A ***standardized approach*** to investigation of healthcare-associated

NTM acquisition in CF patients ***is critical to prevention***. Collecting data on potential healthcare-associated transmission events in a systematic, uniform format will increase the likelihood of being able to create a statistical model to predict instances of increased probability of transmission events. Understanding risk for NTM transmission in the setting of the current IP&C guidelines has the potential to ***significantly decrease risk of healthcare-associated transmission events of NTM among people with CF***.

An overview of the HALT NTM investigation process is outlined (**Figure 4**). NJH IRB approval for the HALT NTM toolkit is active. Currently the CO-RDP receives CF NTM respiratory samples from across the country and performs culture and WGS analysis. Submitting physicians receive a letter acknowledging receipt of the sample; most commonly informing them that the isolate is genetically unique. However, samples initially identified as unique may subsequently be identified as part of a cluster of highly similar isolates when additional strains are added to the database. We will continue to update the Care Centers on the status of their isolates periodically. When WGS related clusters are identified, we will determine if the patients receive care in the same CF Program.

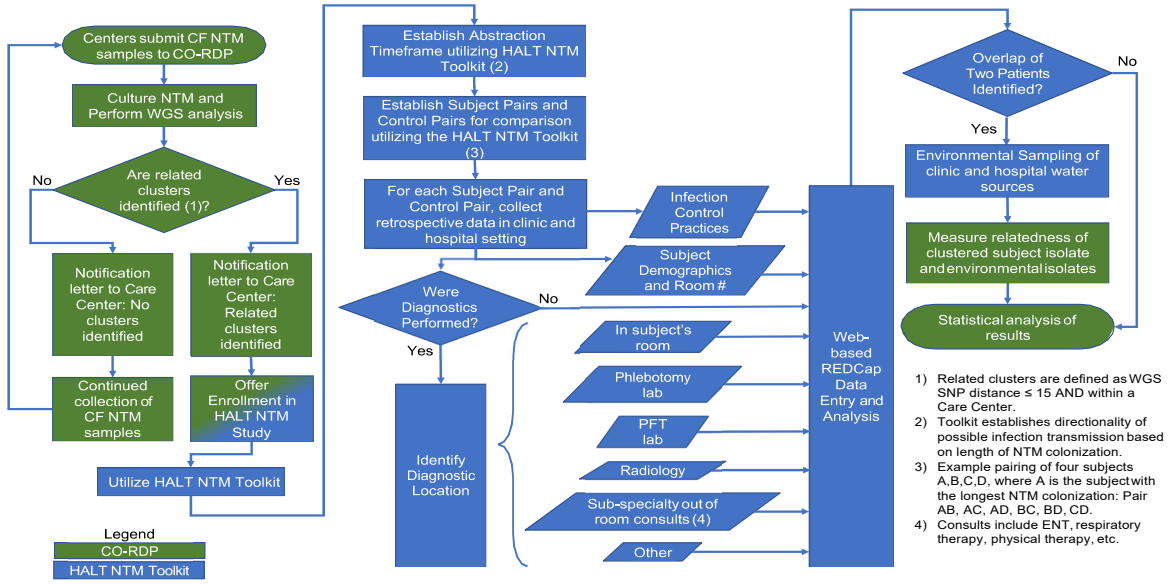

Figure 4: HALT NTM Study Flowchart.

When highly similar isolates are identified within an individual Care Center, a form letter entitled, CO-RDP NTM Isolate Results for CF Care Centers (**Appendix 1**), will be sent to each affected institution. The letter informs the Centers of highly similar NTM isolates among two or more patients within their Center. The letter will also extend the invitation to participate in the HALT NTM study. To put the study in context, we will provide a summary of cluster data available to date, the goals and methods of this study, as well as IRB documents. The invitation to participate in HALT NTM will initiate a simple enrollment process and recommend collaboration with the Center's local IP&C team for investigation into a potential healthcare- associated transmission event. With enrollment, instructions will be provided on how to access the web-based HALT NTM investigation toolkit to facilitate a standardized healthcare-associated NTM investigation and a HALT NTM Toolkit User's Guide will be provided (**Appendix 2**).

The primary function of the HALT NTM abstraction toolkit is to assess an occurrence of overlap between two or more patients identified in the cluster within the CF healthcare setting, defined as CF clinic, hospital and research settings. An overlap is defined as a healthcare setting where two or more CF patients identified in the cluster received care in the same setting for any period of time **within 24 hours**. A point instance of overlap is defined as a specific physical room within the healthcare setting (clinic, hospital, or research area) where two or more CF patients identified in the cluster received care. An example includes the following: Subject 1 and Subject 2 are both seen in clinic on 12/12/16. Subject 1 is in the clinic from 10:00-12:00. Subject 2 is in clinic from 1:00-3:00. In this example, an overlap in clinic is observed between Subject 1 and Subject 2 because two clustered patients attended the same CF clinic on the same day. During this example visit, Subject 1 performs spirometry in PFT laboratory Room 1 at 10:00 on 12/12/16 and Subject 2 performs spirometry in PFT laboratory Room 1 at 2:00 on 12/12/16. In this example, a point instance overlap is observed in the PFT laboratory because two clustered patients were in Room 1 in the PFT laboratory on the same day. Because potential transmission events can be direct or indirect, an overlap does not have to occur at the exact same time on a given day. Additionally, subjects may be observed to have an overlap, but a point instance of overlap may not be identified. In either case, data will be recorded accordingly.

Testing for water biofilm sources in the CF healthcare setting: NTM are known to colonize municipal water systems and have been identified in healthcare facilities.[27-29] NTM are found in free flowing water as well as biofilms on water supply system pipes and both have been implicated in nosocomial outbreaks of infection.[30] Modes of acquisition and transmission of NTM among CF patients remains unclear. A multicenter prevalence study of NTM in 2003 did not implicate nosocomial transmission among CF patients, and molecular analysis at that time revealed almost all patients had unique NTM strains.[31] A more recent study described a biphasic nosocomial outbreak of MAB linked to hospital tap water.[32] The authors attributed the first phase of the outbreak of respiratory MAB to routine care practices using hospital tap water with exposure to the aerodigestive tract in high-risk patients. The second phase outbreak occurred among cardiac surgery patients with invasive MAB infections and was attributed to contaminated heater-cooler units of cardiopulmonary bypass machines. Others have implicated inhalation of aerosolized NTM via showerheads as a possible source of acquisition of infection.[33] A recent study demonstrated that showerheads receiving water from municipal water treatment plants ***harbor clinically-relevant NTM***.[34] On a more global level, Bryant *et al.* reported widespread dominant circulating NTM clones.[11] This worldwide distribution could be explained by ***prevalent*** **environmental genotypes**. Further environmental evaluation of healthcare environments is essential to understanding healthcare-associated environmental risk factors in ***acquisition of NTM infection***.

Biofilms are the preferred samples for isolation of NTM in water systems of all types because the hydrophobic NTM cells preferentially adhere to pipe surfaces where they form biofilms, thus ensuring their persistence. Average colony counts/cm^2 of premise plumbing biofilm samples is between 1,000-15,000 CFU/cm^2, whereas water (suspended) densities are between 10-100 CFU/mL.[35] Thus, the sensitivity of NTM detection is ***orders of magnitude higher for biofilm*** compared to water samples. With advanced molecular analysis and better understanding of previous NTM nosocomial outbreaks, the CO-RDP findings of highly related NTM clusters among CF patients within the same CF Care Centers ***raises concern for nosocomial***

transmission. In order to better understand risk for nosocomial NTM acquisition among CF patients with clonal NTM respiratory isolates, ***determining the relatedness of clustered CF NTM clinical respiratory isolates to environmental water sources in the CF healthcare setting is imperative***.

2.1 Risk / Benefit Assessment

As a retrospective, epidemiologic study there is no anticipated direct benefit to participants. There is minimal risk to subjects in the study.

3 STUDY OBJECTIVES

3.1 Primary Objective

PART A / Epidemiologic Investigation:

The primary objective of the epidemiologic investigation is to facilitate implementation of a stepwise process by which individual Centers perform retrospective epidemiologic evaluation of patients identified by the CO-RDP as part of an NTM cluster.

PART B / Water Biofilm Collection:

The primary objective of water biofilm collection is to determine of NTM strains identified in clusters are related to the strains isolated from water biofilm sources in the CF healthcare setting.

3.2 Secondary Objectives

PART A / Epidemiologic Investigation:

The secondary objectives are to:

- Determine subject overlap:
  Frequency, location and source of overlap within the healthcare system within the 2-year abstraction timeframe.
- Evaluate infection prevention and control (IP&C) measures:
  Use of single patient rooms, healthcare staff gowning and gloving, patients wearing masks, aggressive cleaning procedures between patients, and maintenance of patient-to- patient separation of 6 feet or more within the 2-year abstraction timeframe.

PART B / Water Biofilm Collection:

The secondary objectives are to:

- Culture healthcare-associated water biofilm environmental NTM.
- Evaluate effectiveness of a standardized NTM disease treatment protocol to serve as baseline/reference estimates of endpoints in clinical care and future therapeutic trials.
- Support biomarker development for markers of NTM treatment response through banking of clinical specimens and NTM isolates linked with outcomes data.
- Characterize clinical features of patients achieving treatment success compared to those unresponsive to treatment.

4 STUDY DESIGN

4.1 Study Overview

PART A / Epidemiologic Investigation:

This study will retrospectively review and collect data from patients with CF and culture positive for an NTM isolate falling in a highly-related cluster, identified by the CO-RDP, within a single CF Care Center. Two control subjects will be identified by the CO-RDP for every subject falling within a cluster. Retrospective epidemiologic data collection and input into REDCap is detailed (**Appendix 1 and 2)**. Up to 20 Care Centers will be offered enrollment.

PART B / Water Biofilm Collection:

This study will also collect and analyze water biofilms from a variety of water sources in the healthcare setting including sink faucets, showerheads, shower hoses, ice machines, drinking fountains, and decorative water features. Environmental sampling instructions are detailed (**Appendix 3)**.

5 CRITERIA FOR EVALUATION

5.1 Primary Endpoint

PART A / Epidemiologic Investigation:

Identification of a shared healthcare-associated source(s) between patients in a Care Center with identical NTM isolates.

PART B / Water Biofilm Collection:

Identification of clinically-relevant NTM isolates and prevalent environmental genotypes with in healthcare setting water biofilms.

5.2 Secondary Endpoints

PART A / Epidemiologic Investigation:

The secondary endpoints are:

- Adherence to CF IP&C guidelines.
- Characterization of points of patient overlap.

PART B / Water Biofilm Collection:

The secondary endpoints are:

- Incidence and prevalence of NTM species/subspecies within healthcare water biofilms.
- Determine if biofilm isolates are highly related to isolates recovered from patients.

6 PARTICIPANT SELECTION

6.1 Study Population

Participants with a diagnosis of CF who meet all of the inclusion and none of the exclusion criteria will be eligible for participation in this study.

6.2 Inclusion Criteria

PART A / Epidemiologic Investigation:

1. Male or female participant of any age at enrollment Diagnosis of CF consistent with the 2017 CFF Guidelines.
2. NTM-positive for a **species or sub-species** in the 2 years prior to enrollment that has never been treated or current Denver single-center study participant.

PART B / Water Biofilm Collection:

N/A

6.3 Exclusion Criteria

PART A / Epidemiologic Investigation:

N/A

PART B / Water Biofilm Collection:

N/A

6.4 Study Specific Tolerance for Inclusion/Exclusion Criteria

N/A

6.5 Screen Fail Criteria

N/A

7 CONCURRENT MEDICATIONS

7.1 Allowed Medications and Treatments

No available therapy for CF is restricted.

7.2 Prohibited Medications and Treatments

N/A

8 STUDY TREATMENTS

Part A (Epidemiologic Investigation) is retrospective collection of epidemiologic data. Part B (Water Biofilm Collection) is exclusively environmental sampling and does not include subject involvement.

9 STUDY PROCEDURES AND GUIDELINES

REDCap data collection is detailed (Appendix 1 and Appendix 2 - Epidemiologic Investigation). Environmental sampling instructions are detailed (Appendix 3).

9.1 Clinical Assessments

9.1.1 Demographics and CFF Registry ID

Demographic information (date of birth, sex, race, zip code) will be recorded. CFF Registry number will be recorded for participating CF participants.

9.1.2 CF Diagnosis

CF diagnosis will be confirmed by the participating Care Center.

9.1.3 NTM History

Results from molecular analysis performed at National Jewish Health Mycobacteriology and Pharmacokinetics Laboratory will be recorded.

9.1.4 NTM Disease Diagnosis Evaluation

Investigators will record if a diagnosis of CF NTM disease is met based on the US CFF and European CF Society consensus recommendations for the management of NTM in individuals with CF.

9.1.5 NTM Treatment

Investigators will record if the subject underwent or is undergoing treatment of CF NTM disease.

9.1.6 Drug Toxicity Monitoring

N/A

9.2 Clinical Laboratory Measurements

N/A

9.3 Research Laboratory Measurements

N/A

10 EVALUATIONS BY VISIT

Not applicable.

11 ADVERSE EXPERIENCE REPORTING AND DOCUMENTATION

Not applicable. Adverse Events and Serious Adverse Experience will not be reported for this retrospective study.

12 DISCONTINUATION AND REPLACEMENT OF PARTICIPANTS

12.1 Early Withdrawal of Participants from the Study

N/A.

12.2 Replacement of Participants

N/A

13 PROTOCOL VIOLATIONS

A protocol violation occurs when the participant, investigator, or the Sponsor fails to adhere to significant protocol requirements affecting the eligibility criteria and primary endpoint criteria.

Failure to comply with Good Clinical Practice (GCP) guidelines may also result in a protocol violation.

There will be no protocol violations. We will monitor for protocol deviations. If deviations are identified, we will request modifications as necessary to meet protocol guidelines.

14 DATA SAFETY MONITORING

N/A

15 STATISTICAL METHODS AND CONSIDERATIONS

15.1 General Considerations

For each of the primary and secondary outcomes, biostatistical analysis of the multicenter data will be completed using both qualitative and quantitative approaches. We will identify a set of variables used to assess degree of proximity of subjects in time and/or space. For example, two subjects with neighboring rooms at a hospital would have close proximity, but proximity would be increased for two subjects at a facility that had longer overlap time, relative to a pair of subjects that had shorter overlap. We will explore creating composite measures of quantitative proximity that incorporate multiple variables. Qualitative measures of proximity, such as location type (testing laboratory, patient room) will also be assessed. Several testing approaches will be used to compare the relationship between similarity of strains and degree of proximity of subjects. In the simplest form, descriptive statistics will be computed for proximity variables, for pairs of subjects who are determined to have similar strains versus those who have dissimilar strains, and multivariate or 2-sample t-tests will be performed to test for differences between these groups. More advanced modeling will be performed to determine the relationship between the degree of similarity in strains with the proximity and subject characteristic variables; multiple linear regression will be used when considering continuous strain similarity, while multiple logistic regression will be used when considering subjects with dichotomized (similar/dissimilar) strains to other subjects.

15.1.1 Data Sets Analyzed

All analyses will be based on the group of subjects that are identified by the CO-RDP as having NTM isolates, defined as highly similar strains at the genomic level, harbored by two or more patients within a single Care Center.

15.2 Demographic and Baseline Characteristics

The following baseline demographic and clinical characteristics will be reported: age, gender, race, ethnicity, zip code, and NTM sub/species.

15.3 Analysis of Primary Endpoint

PART A / Epidemiologic Investigation:

The primary endpoint will characterize the origins and prevalence of healthcare-associated patient-to-patient transmission of NTM among different CF Care Centers. We will further characterize the source(s) of direct or indirect transmission of NTM within an individual CF healthcare setting. We will identify a set of variables used to assess degree of proximity of subjects in time and/or space. For example, two subjects with neighboring rooms at a hospital would have close proximity, but proximity would be increased for two subjects at a facility that had longer overlap time, relative to a pair of subjects that had shorter overlap. We will explore creating composite measures of quantitative proximity that incorporate multiple variables. Qualitative measures of proximity, such as location type (testing laboratory, patient room) will also be assessed. Several testing approaches will be used to compare the relationship between similarity of strains and degree of proximity of subjects. In the simplest form, descriptive statistics will be computed for proximity variables, for pairs of subjects who are determined to have similar strains versus those who have dissimilar strains, and multivariate or 2-sample t-tests will be performed to test for differences between these groups. More advanced modeling will be performed to determine the relationship between the degree of similarity in strains with the proximity and subject characteristic variables; multiple linear regression will be used when considering continuous strain similarity, while multiple logistic regression will be used when considering subjects with dichotomized (similar/dissimilar) strains to other subjects.

PART B / Water Biofilm Collection:

The primary endpoint will characterize clinically-relevant NTM found in water biofilms within CF healthcare settings and determine if the biofilm isolates are highly related to isolates recovered from patients.

15.4 Analysis of Secondary Endpoints

PART A / Epidemiologic Investigation:

The secondary endpoints will characterize CF Center adherence to the CF IP&C guidelines as well as characterize points of patient overlap. Basic summaries of points of potential transmission overlap will be provided.

PART B / Water Biofilm Collection:

The incidence and prevalence of NTM species and subspecies within healthcare water biofilms will be characterized. Comparisons of NTM biofilm isolates and highly related patient isolates will be provided.

15.5 Interim Analysis

No formal interim analysis involving a data monitoring committee (DMC) is planned. However, annual reports summarizing key variables related to site enrollment and adherence to protocol

will be generated. These reports will help guide protocol revisions. Details of the planned analysis for these annual reports can be found in the Statistical Analysis Plan (SAP).

15.6 Sample Size

Up to 20 CF Care Centers are eligible for enrollment. Sample size will be determined based on CO-RDP WGS finding of highly related NTM species and subspecies among patients receiving care at an individual CF Care Center.

16 DATA COLLECTION, RETENTION AND CLINICAL MONITORING

16.1 Data Collection Instruments

Study personnel at each site will enter data from the electronic medical record and CF Registry corresponding to a participant's visit into the protocol-specific electronic REDCap database when the information corresponding to that visit is available. Participants will not be identified by name in the study database to be collected by the Sponsor (or designee), but will be identified by a site number, participant number and initials.

For all REDCap data collection, the time and date stamp tracks the person entering or updating data and creates an electronic audit trail.

The Investigator is responsible for all information collected on participants enrolled in this study. All data collected during the course of this study must be reviewed and verified for completeness and accuracy by the Investigator. At the completion of the study, a copy of the REDCap data will be provided to the site to be retained at the Investigator's site.

The data may be monitored by the CFF at any time during the study.

16.2 Data Management Procedures

The National Jewish Health Research Informatics Services Core will be used as a central location for data processing and management. REDCap, a software toolset and workflow methodology for electronic collection and management of research and clinical trial data, was developed by Vanderbilt University, with collaboration from a consortium of institutional partners. REDCap data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the Research Informatics Services Core. The iterative development and testing process results in a well-planned data collection strategy for individual studies. REDCap servers are housed in a local data center at National Jewish Health and all web-based information transmission is encrypted. REDCap was developed specifically around HIPAA-security guidelines and is recommended to National Jewish Health researchers by both our Privacy Office and Institutional Review Board. REDCap currently supports 240+ academic/non-profit consortium partners on six continents and over 26,000 research end-users (www.project- redcap.org).

Data Quality Control and Reporting

After data have been entered into the study database, data validation checks will be applied on a regular basis. As part of data analysis, Matthew Strand, the lead biostatistician on the study, will monitor for data outliers and will perform data control. If data entry errors are encountered, the

study database will be updated in accordance with the resolved queries. All changes to the study database will be documented in an audit trail.

16.3 Security and Archival of Data

REDCap is a secure, web-based application designed to support data capture for research studies. REDCap is maintained by the REDCap Consortium which is comprised of over 3,500 institutional partners including National Jewish Health, and is administrated locally by the National Jewish Health Research Informatics Services.

REDCap utilizes as suite of features that support HIPAA compliance. Access to the database requires user authentication with password. Data is accessed based on the individual's role on the project. Logging and audit trails are maintained on all data interactions. All data is stored on a secure server, and backups are encrypted. Automatic backup of the servers are performed twice daily.

16.4 Availability and Retention of Investigational Records

The Investigator must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors upon request. Hard copy files will not be created.

All study information will be maintained in REDCap for a period of 5 years after database lock. There may be other circumstances for which the Sponsor is required to maintain study records and, therefore, the Sponsor should be contacted prior to removing study records for any reason.

16.5 Monitoring

The study PIs and the CFF are authorized to monitor the study. By signing this protocol, the Investigator grants permission to the Sponsor (or designee), and appropriate regulatory authorities to conduct on-site monitoring and/or auditing of all appropriate study documentation. Monitoring visits will be conducted by representatives of the Sponsor according to the U.S. CFR 21 Part 312 and ICH Guidelines for GCP (E6) and to ensure investigator compliance to 21 CFR Parts 50, 56 and 312 and to GCP.

16.6 Participant Confidentiality

In order to maintain participant confidentiality, only a site number, participant number and participant initials will identify all study participants in REDCap. The participant's CFF patient registry number will also be collected. Additional participant confidentiality issues (if applicable) are covered in the Clinical Study Agreement.

17 ADMINISTRATIVE, ETHICAL, REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a coded number and initials only. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the FDA.

The Investigator must also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

17.1 Protocol Amendments

Any amendment to the protocol will be written by the Sponsor. Protocol amendments cannot be implemented without prior written IRB/IEC approval except as necessary to eliminate immediate safety hazards to participants. A protocol amendment intended to eliminate an apparent immediate hazard to participants may be implemented immediately, provided the IRBs are notified within 5 working days.

17.2 Institutional Review Boards and Independent Ethics Committees

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. Serious adverse experiences regardless of causality will be reported to the IRB/IEC in accordance with the standard operating procedures and policies of the IRB/IEC, and the Investigator will keep the IRB/IEC informed as to the progress of the study. The Investigator will obtain assurance of IRB/IEC compliance with regulations.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning participant recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. The IRB/IEC's unconditional approval statement will be transmitted by the Investigator to the Sponsor or designee prior to the shipment of study supplies to the site. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes may not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the participants or when the change(s) involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved should be obtained.

The IRB/IEC must be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the participants of the conduct of the study; an annual update and/or request for re-approval; and when the study has been completed.

17.3 Informed Consent Form

N/A

17.4 Consent for Collection and Use of CFF Registry ID Number

To facilitate possible future evaluation of retrospective and prospective information from all participants in this study, the participant's CFF Registry ID number will be collected. The CFF registry collects data on all CF patients who consented to participate in the CFF registry and who are followed at CFF-accredited care centers. The registry data includes information from clinical

encounters, hospitalizations courses of antibiotics, and year-end surveys. Data also include microbiology results, spirometry results, CF genotype and other information. If specific consent is given to collect this number, the participant's CF registry number will be recorded in the CRF.

17.5 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the study Sponsor and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

17.6 Investigator Responsibilities

By signing the Agreement of Investigator form, the Investigator agrees to:

1. Conduct the study in accordance with the protocol and only make changes after notifying the Sponsor (or designee), except when to protect the safety, rights or welfare of participants.
2. Personally conduct or supervise the study (or investigation).
3. Ensure that the requirements relating to obtaining informed consent and IRB review and approval meet federal guidelines, as stated in § 21 CFR, parts 50 and 56.
4. Ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations in meeting the above commitments.
5. Maintain adequate and accurate records in accordance with §21 CFR 312.62 and to make those records available for inspection with the Sponsor (or designee).
6. Ensure that an IRB that complies with the requirements of §21 CFR part 56 will be responsible for initial and continuing review and approval of the clinical study.
7. Promptly report to the IRB and the Sponsor (or designee) all changes in the research activity and all unanticipated problems involving risks to participants or others (to include amendments and IND safety reports).
8. Seek IRB approval before any changes are made in the research study, except when necessary to eliminate hazards to the participants.
9. Comply with all other requirements regarding the obligations of clinical investigators and all other pertinent requirements listed in § 21 CFR part 312.

18 REFERENCES

1. Qvist T, Pressler T, Hoiby N, Katzenstein TL. Shifting paradigms of nontuberculous mycobacteria in cystic fibrosis. Respir Res 2014;15:41.
2. Floto RA, Olivier KN, Saiman L, et al. US Cystic Fibrosis Foundation and European Cystic Fibrosis Society consensus recommendations for the management of non-tuberculous mycobacteria in individuals with cystic fibrosis. Thorax 2016;71 Suppl 1:i1-22.
3. Adjemian J, Olivier KN, Prevots DR. Epidemiology of Pulmonary Nontuberculous Mycobacterial Sputum Positivity in Patients with Cystic Fibrosis in the United States, 2010-2014. Ann Am Thorac Soc 2018;15:817-26.
4. Primm TP, Lucero CA, Falkinham JO, 3rd. Health impacts of environmental mycobacteria. Clin Microbiol Rev 2004;17:98-106.
5. Falkinham JO, 3rd. Nontuberculous mycobacteria from household plumbing of patients with nontuberculous mycobacteria disease. Emerg Infect Dis 2011;17:419-24.
6. Honda JR, Hasan NA, Davidson RM, et al. Environmental Nontuberculous Mycobacteria in the Hawaiian Islands. PLoS Negl Trop Dis 2016;10:e0005068.
7. Prevots DR, Adjemian J, Fernandez AG, Knowles MR, Olivier KN. Environmental risks for nontuberculous mycobacteria. Individual exposures and climatic factors in the cystic fibrosis population. Ann Am Thorac Soc 2014;11:1032-8.
8. Aitken ML, Limaye A, Pottinger P, et al. Respiratory outbreak of Mycobacterium abscessus subspecies massiliense in a lung transplant and cystic fibrosis center. Am J Respir Crit Care Med 2012;185:231-2.
9. Bryant JM, Grogono DM, Greaves D, et al. Whole-genome sequencing to identify transmission of Mycobacterium abscessus between patients with cystic fibrosis: a retrospective cohort study. Lancet 2013;381:1551-60.
10. Johnston DI, Chisty Z, Gross JE, Park SY. Investigation of Mycobacterium abscessus outbreak among cystic fibrosis patients, Hawaii 2012. J Hosp Infect 2016;94:198-200.
11. Bryant JM, Grogono DM, Rodriguez-Rincon D, et al. Emergence and spread of a human-transmissible multidrug-resistant nontuberculous mycobacterium. Science 2016;354:751-7.
12. Colorado Cystic Fibrosis Research & Development Program. National Jewish Health. (Accessed Sept 2, 2018, at https://www.nationaljewish.org/cocfrdp.)
13. Epperson LE, Hasan NA, Davidson RM, et al. The Colorado Research and Development Program Molecular and Genomics Core: Surveillance for Nontuberculous Mycobacteria Transmission or Shared Environmental Exposure in US Cystic Fibrosis Patients. Poster session presented at: The 31st Annual North American Cystic Fibrosis Conference; 2017 Nov 2-4; Indianapolis, IN.
14. Healthcare-Associated Infection (HAI) Outbreak Investigation Toolkit. Centers for Disease Control and Prevention, 2013. (Accessed Sept 2, 2018, at https://www.cdc.gov/hai/outbreaks/outbreaktoolkit.html.)
15. Lipner EM, Knox D, French J, Rudman J, Strong M, Crooks JL. A Geospatial Epidemiologic Analysis of Nontuberculous Mycobacterial Infection: An Ecological Study in Colorado. Ann Am Thorac Soc 2017;14:1523-32.
16. Lande L, Alexander DC, Wallace RJ Jr, Kwait R, Iakhiaeva E, Williams M, et al. Mycobacterium avium in community and household water, suburban Philadelphia, Pennsylvania, USA, 2010-2012. Emerg Infect Dis 2019 Mar 14.

17. Cystic Fibrosis Foundation Patient Registry 2016 Annual Data Report. Bethesda, MD: Cystic Fibrosis Foundation; 2017.
18. Cystic Fibrosis Foundation Patient Registry 2017 Annual Data Report. Bethesda, MD: Cystic Fibrosis Foundation; 2018.
19. Hasan NA, Davidson RM, Epperson LE, et al. The Genomic Epidemiology of *Mycobacterium avium complex* vs *Mycobacterium abscessus* in United States Cystic Fibrosis Patients. Poster session presented at: The 31st Annual North American Cystic Fibrosis Conference; 2017 Nov 2-4; Indianapolis, IN.
20. Martiniano SL, Davidson RM, Nick JA. Nontuberculous mycobacteria in cystic fibrosis: Updates and the path forward. Pediatr Pulmonol 2017;52:S29-S36.
21. Tortoli E, Kohl TA, Trovato A, et al. Mycobacterium abscessus in patients with cystic fibrosis: low impact of inter-human transmission in Italy. Eur Respir J 2017;50.
22. Adjemian J, Olivier KN, Seitz AE, Holland SM, Prevots DR. Prevalence of nontuberculous mycobacterial lung disease in U.S. Medicare beneficiaries. Am J Respir Crit Care Med 2012;185:881-6.
23. Gross JE. *Mycobacterium abscessus* Epidemic Among Cystic Fibrosis Patients. Poster session presented at: The 27th Annual North American Cystic Fibrosis Conference; 2013 Oct 17-19; Salt Lake City, UT.
24. Gross JE. Overcoming Barriers to Infection Control in Patients With Cystic Fibrosis. Oral presentation at: The 28th Annual North American Cystic Fibrosis Conference; 2014 Oct 9- 11; Atlanta, GA.
25. Gross JE. Healthcare-Associated Transmission of Nontuberculous mycobacteria Among Patients With Cystic Fibrosis. Oral presentation at: The 32nd Annual North American Cystic Fibrosis Conference; 2018 Oct 18-20; Denver, CO.
26. Saiman L, Siegel JD, LiPuma JJ, et al. Infection prevention and control guideline for cystic fibrosis: 2013 update. Infect Control Hosp Epidemiol 2014;35 Suppl 1:S1-S67.
27. Williams MM, Armbruster CR, Arduino MJ. Plumbing of hospital premises is a reservoir for opportunistically pathogenic microorganisms: a review. Biofouling 2013;29:147-62.
28. Shin JH, Lee EJ, Lee HR, et al. Prevalence of non-tuberculous mycobacteria in a hospital environment. J Hosp Infect 2007;65:143-8.
29. du Moulin GC, Stottmeier KD, Pelletier PA, Tsang AY, Hedley-Whyte J. Concentration of Mycobacterium avium by hospital hot water systems. JAMA 1988;260:1599-601.
30. Wallace RJ, Jr., Brown BA, Griffith DE. Nosocomial outbreaks/pseudo-outbreaks caused by nontuberculous mycobacteria. Annu Rev Microbiol 1998;52:453-90.
31. Olivier KN, Weber DJ, Wallace RJ, Jr., et al. Nontuberculous mycobacteria. I: multicenter prevalence study in cystic fibrosis. Am J Respir Crit Care Med 2003;167:828-34.
32. Baker AW, Lewis SS, Alexander BD, et al. Two-Phase Hospital-Associated Outbreak of Mycobacterium abscessus: Investigation and Mitigation. Clin Infect Dis 2017;64:902-11.
33. Thomson R, Tolson C, Carter R, Coulter C, Huygens F, Hargreaves M. Isolation of nontuberculous mycobacteria (NTM) from household water and shower aerosols in patients with pulmonary disease caused by NTM. J Clin Microbiol 2013;51:3006-11.
34. Gebert MJ, Delgado-Baquerizo M, Oliverio AM, et al. Ecological Analyses of Mycobacteria in Showerhead Biofilms and Their Relevance to Human Health. MBio 2018;9.
35. Falkinham JO, 3rd, Norton CD, LeChevallier MW. Factors influencing numbers of Mycobacterium avium, Mycobacterium intracellulare, and other Mycobacteria in drinking water distribution systems. Appl Environ Microbiol 2001;67:1225-31.

36. Martiniano SL, Sontag MK, Daley CL, Nick JA, Sagel SD. Clinical significance of a first positive nontuberculous mycobacteria culture in cystic fibrosis. Ann Am Thorac Soc 2014;11:36-44.

APPENDIX 1 COLORADO-RESEARCH AND DEVELOPMENT PROGRAM NTM ISOLATE LETTER

National Jewish Health®
Advanced Diagnostic Laboratories

October 30, 2019

[redacted]

Dear Dr. ,

The CF Foundation-funded Colorado Research and Development Program (RDP) is engaged in a nationwide study of the genetic characterization of nontuberculous mycobacterium (NTM) across United States CF Care Centers. We recently confirmed identification of NTM isolates from the CF patients indicated below, who receive care in your CF center. We have genotyped these isolates by using *rpoB* gene sequencing (clinical only, not for research), as well as whole genome sequencing, which provided a genetic profile (DNA fingerprint), enablingus to determine similarity between strains.

This analysis indicates that the patients listed below are infected with *Mycobacterium abscessus* ssp. *abscessus* and share a highly similar strain that could be due to a shared environmental exposure or possibly person-to-person transmission.

Patient Name	Date of Birth
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

The information above is for research purposes only. If you are interested in conducting an independent, internal investigation for a shared healthcare-associated source(s) between two or more patients in your Center, please contact Jane Gross, MD at GrossJane@njhealth.org to access the Healthcare-Associated Links in Transmission of Nontuberculous Mycobacteria in Patients with Cystic Fibrosis (HALT NTM) toolkit.

If you have questions about this report please contact the Clinical Research Coordinator, Adrah Levin, MPH, at LevinA@njhealth.org or (303) 398-1407. You can also contact the RDP Program Director, Jerry Nick, MD, at NickJ@njhealth.org or at (303) 398-1579. If one of more of these patients are no longer under your care, or if you believe you have received this letter in error, please contact us so we can update our records.

Sincerely,

Charles L Daley, MD
Director of RDP Culture and Biorepository Core
Chief of Division of Mycobacterial and Respiratory Infections
DaleyC@NJHealth.org

Previously reported patients sharing similar strains

Mycobacterium abscessus, subsp. abscessus (reported 5/2018)

Patient Name	Date of Birth
[redacted]	[redacted]
[redacted]	[redacted]

Mycobacterium abscessus, subsp. abscessus (reported 1/2019)

Patient Name	Date of Birth
[redacted]	[redacted]
[redacted]	[redacted]

Mycobacterium abscessus ssp. *massiliense* (reported 1/2019)

Patient Name	Date of Birth
[redacted]	[redacted]
[redacted]	[redacted]

Mycobacterium intracellulare (reported 1/2019)

Patient Name	Date of Birth
[redacted]	[redacted]
[redacted]	[redacted]

Mycobacterium avium (reported 1/2019)

Patient Name	Date of Birth
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Mycobacterium abscessus ssp. *abscessus* (reported 1/2019)

Patient Name	Date of Birth
[redacted]	[redacted]
[redacted]	[redacted]

Mycobacterium avium (reported 1/2019)

Patient Name	Date of Birth
[redacted]	[redacted]
[redacted]	[redacted]

Mycobacterium abscessus ssp. *abscessus* (reported 9/2019)

Patient Name	Date of Birth
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

National Jewish Health®
Advanced Diagnostic Laboratories

Mycobacterium abscessus ssp. *abscessus* (reported 9/2019)

Patient Name	Date of Birth
[redacted]	[redacted]
[redacted]	[redacted]

Mycobacterium abscessus ssp. *abscessus* (reported 9/2019)

Patient Name	Date of Birth
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Mycobacterium abscessus ssp. *abscessus* (reported 9/2019)

Patient Name	Date of Birth
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

1400 Jackson Street | Denver, Colorado 80206
njlabs.org | 800.550.6227 (toll-free) | 800.652.9556 (fax)

APPENDIX 2 REDCAP USER GUIDE

So, you've received a letter from National Jewish Health Advanced Diagnostic Laboratories (NJH ADx) saying you have patients at your center with a highly similar strain of NTM. Now what?

1. Contact Dr. Gross at NJH. She will help you with initial questions regarding study design and study start-up, including IRB and database requirements.
2. For NJH REDCap access, you will be sent a link to complete REDCap training. You will be asked the following:
    - For the question "Are you an employee of National Jewish Health?" select No.
    - Explain your affiliation as "HALT NTM"
    - National Jewish Health Sponsor is "Jane Gross"
    - For department, indicate your CF center/institution name.
    - Complete the rest as indicated.
    - Email Dr. Gross or Katie once you've been given access so you can be added to the project.
3. Now you have access to the database. Log on to REDCap. On the welcome screen, open the HALT NTM project.
4. On the left hand side under Applications, open the File Repository. Download the HALT NTM cheat sheet.
5. Print a HALT NTM cheat sheet for each unique pair:
    - If there are two patients listed on your letter, you will have one unique pair.
    - If there are three patients listed on your letter, you will have three unique pairs.
    - If there are four patients listed on your letter, you will have six unique pairs.
    - This is because the date ranges are compared between two subjects and is unique for each pair.
6. Complete the first six lines on the HALT NTM cheat sheet for each unique pair. You will need to refer to it during pairing and data extraction.
7. Back in REDCap, on the left hand side under Data Collection, click Add / Edit Records. On the Add/Edit Records page, click on the button Add new record. You will be taken to the Record Home Page. There will be a **NEW** Study ID **XXX-XX**. Record that number (**XXX-XX**) on the **HALT NTM cheat sheet**. It is auto-generated by REDCap. You will need it later.
8. The first Form to complete is Demographics. Open the form by clicking on the circle under Demographics. Near the top of the form under the header **Pairs Survey** is a link to the "**Finding Pairs survey**." Click on the link. You will be taken to an interactive tool that will help you determine if this subject is "subject 1" or "subject 2" in this pairing.
9. Using the letter from NJH ADx and the HALT NTM cheat sheet, answer the questions. This will give you the pair number and subject number for the patients. Record those numbers on the HALT NTM cheat sheet. You may also choose to click Submit and then Download to get a printout of your survey responses for your record.

10. Back on the Demographic form, complete the questions for the first subject in the pair. Have the HALT NTM cheat sheet handy. You will use it to enter the Pair Number, Subject Number, culture dates, and isolate ID into REDCap. Enter as much data as you can. Once complete, click on Save & Exit Form button.
11. Repeat Steps 6-9 for the second subject in the pair.
12. Now that you have the demographics and some background information entered for both subjects in a pair, the next Form to complete is Compare. You can access it from the Record Status Dashboard **or** from Add/Edit Records listed under Data Collection on the left hand side.
13. Using the HALT NTM cheat sheet, answer "For this pairing, is this Subject 1 or Subject 2?" Depending on your answer, you will be asked to enter a date for the **OTHER PATIENT** in this pairing. Be sure to read the questions carefully and enter the correct date.
14. Once the date has been entered, a box will pop-up with the Extraction Date Range. Record this on the HALT NTM cheat sheet.
15. Now for the digging. In order to see if your pair of patients had any overlap, you will need to enter dates for Inpatient hospitalizations, Outpatient Clinic visits, and Research Unit visits. Only record visits that fall within the Extraction Date Range.
16. Keep digging! You've completed the extraction for the first subject in the pair. Now repeat Steps 12-15 for the second subject in the pair.
17. Once you have completed the Compare Form (all the date extractions) for a pair of subjects, you are ready to see if there is any overlap between these two patients. Click on "Compare Dates" on the left side under Project Bookmarks. You will be asked to enter the internal record id (**XXX-XX)** for the two subjects in the pair you are comparing.
18. Take a deep breath and click "Compare subjects." The program will let you know if there is any overlap in dates for Inpatient, Clinic, and/or Research Visits. If there is, REDCap will mark those forms as **red circles** on the Record Status Dashboard. If there is no overlap, the circles will stay grey and you are done with this pair.
19. However, if you have **red circles** on the Record Status Dashboard, it is time to dig again. Clicking on a **red circle** will take you to an Overlap Form – there is one form for each location the program found date overlaps. The top of the form is uneditable, but you are able to see the Patient Name, Pair Number, Subject Number, and Date Range for the detailed data extraction. Use this information to complete the questions as best you can for each visit overlap (**red circle**). The more accurate you are able to be, the better the analysis can be.
20. Once done, change status to "Complete," and click "Save & Exit". The status color changes to **green** on the Record Status Dashboard. You are done.

Feel free to contact Katie or Dr. Gross for help along with way or if you have any comments on this user guide or the REDCap project.

Katie Poch
pochk@njhealth.org
303-398-1255

Dr. Jane Gross
grossjane@njhealth.org
303-270-2333

APPENDIX 3 ENVIRONMENTAL SAMPLING PROTOCOL

General Environmental Sampling Instructions

LOCATION RECORDING

Record your location at the time of sampling using GPS. Record each sampling site using a Google Maps on iPhone or Android. For more information:
https://www.wikihow.com/Get-Latitude-and-Longitude-from-Google-Maps

Record GPS similar to the following and out to more than 5 significant digits:
NJ GPS LOCATION: 39°44'23.2"N 104°56'33.6"W

SINK FAUCET SAMPLING

This protocol can be applied to sample water biofilms from kitchen, bathroom, or outdoor sink faucets.

BEFORE SAMPLING:

1. Faucet should be as dry as possible
2. Avoid sampling a faucet if it has been recently used or is wet
3. If faucet is wet, DO NOT dry off. If possible, return to faucet at another time

SAMPLING

1. Identify the sink faucet and take a picture of it using your cell phone or camera.

2. Obtain one of the sterile dual-tipped Flock swabs.

3. Identify the **point of the sink faucet where water exits**. This is where the sample should be taken.

Note: Depending on the faucet, there might be a metal mesh on the faucet head similar the image to the right ("Step 4 under view"). If so, sample the mesh.

Note: Depending on the faucet, there might be an open pipe which water will directly flow out of. If so, sample up into the pipe.

Step 4
Top view

Step 4
Under view

4. Carefully open a new swab package. To do so, carefully pry open the swab package. Remove the swab by grabbing the RED handle and avoid laying the swab down on non-sterile surfaces; keep the swab sterile! **Be careful not to touch anywhere along the swab and only handle the swab by the red handle.**

5. *If mesh sampling* - Firmly and thoroughly swab the **point on the sink faucet where water exits** in a back and forth motion 10 times as well as a circular motion - imagine that you are using the swab to "clean" the surface as completely as possible. Twist the swab over and sample the same surface 10 more times to ensure this side of the swab has also collected biofilms. Avoid moving your hand onto the white applicator stick.

OR

If open pipe sampling – Insert the swab into the pipe and swab the sides of the pipe using a circular, firm stirring motion - similar to stirring your coffee – 10 times. Twist the swab over and sample the same surface 10 more times to ensure all sides of the swab has collected pipe biofilm. Avoid moving your hand onto the white applicator stick.

6. Once you have collected the sample, twist and remove the cap from the sterile collection tube that remains in the swab package and CAREFULLY place the soiled swab into the transport tube, being careful not to touch the swab tips with your fingers.

7. Cap tightly. Label the tube with a Sharpie or pre-prepared label that contains at a minimum the following information: the date, your initials, sample ID, and sample type (*i.e.*, bathroom sink mesh or hospital room sink faucet pipe).

8. Place labeled tubes in a ziploc bag and refrigerate samples until processing.

DUST SAMPLING

1. Locate a dusty area at the sampling site – sample similar sites in all rooms of NJH and SJH.
2. Before sampling, take a representative picture of the dusty area using your cell phone or camera.
3. Obtain one of the sterile dual-tipped Flock swabs.
4. Carefully open a new swab package. To do so, carefully pry open the swab package. Remove the swab by grabbing the RED handle and avoid laying the swab down on non-sterile surfaces; keep the swab sterile! **Be careful not to touch anywhere along the swab and only handle the swab by the red handle tip.**
5. Swab across the dusty surface using a back and forth motion 10 times, imagining that you are using the swab to clean the surface and remove as much dust as possible (refer to the image below). It often helps to rotate the tip of the swab as you brush it across the surface to make sure all sides of the swab get coated with visible dust.
6. Once you have collected the sample, twist and remove the cap from the sterile collection tube that remains in the swab package and CAREFULLY place the soiled dust swab into the transport tube, being careful not to touch the swab tips with your fingers.
7. Cap tightly. Label the tube with a Sharpie or pre-prepared label that contains at a minimum the following information: the date, your initials, sample ID, and sample type *(i.e.,* dust).
8. Place labeled tubes in a ziploc bag and refrigerate samples until processing.

SAMPLING SHOWER HOSES AND SHOWERHEADS

HOW TO SAMPLE THE HOSE (if it has a removable showerhead):

1. Take a representative picture of the end of the showerhead system similar to what is shown in the image using your cell phone or camera
2. *Carefully* twist and remove the showerhead from the shower hose at the connection point labeled "1" on the image to the left. **Do not place the showerhead or hose down**; keep holding both in one of your hands.
3. Obtain **two** of the sterile dual-tipped Flock swabs.
4. Carefully open a new swab package. To do so, carefully pry open the swab package. Remove the swab by grabbing the RED handle and avoid laying the swab down on non-sterile surfaces; keep swab sterile! **Be careful not to touch anywhere along the swab and only handle the swab by the red handle tip.**
5. Firmly and thoroughly, swab the inner portion of the hose at point "2" as shown on the image above. Using a "stirring" motion move the swab in and around the hose opening - imagining that you are using the swab to clean the surface or motion similar to stirring your coffee. Rotate the swab as you swirl to ensure all portions of the swabs have been exposed to biofilms. Rotate and swab 10 times. Avoid moving your hand onto the white applicator stick.
6. Once you have collected the sample from the hose, the hose can now be placed down into the tub or onto a counter. **However, keep holding the showerhead**. Twist and remove the cap from the sterile collection tube that remains in the swab package and CAREFULLY place the soiled swab into the transport tube, being careful not to touch the swab tips with your fingers.
7. Cap tightly. Label the tube with a Sharpie or pre-prepared label that contains at a minimum the following information: the date, your initials, sample ID, and sample type (*i.e.,* showerhead (SHB) hose).
8. Place labeled tubes in a ziploc bag and refrigerate swab until processing.

HOW TO SAMPLE THE SHOWERHEAD FACE:

1. Carefully open a new swab package. To do so, carefully pry open the swab package. Remove the swab by grabbing the RED handle and avoid laying the swab down on non-sterile surfaces; keep swab sterile! **Be careful not to touch anywhere along the swab and only handle the swab by the red handle tip.**
2. Firmly and thoroughly, swab the face of the showerhead (the side from which the water would emerge) at point "3" as shown on the image above. Move the swab back and forth and around the entire surface of the showerhead face 10 times in a circular motion - imagining that you are using the swab to clean the surface as completely as possible. Don't forget to get into the grooves on the showerhead face. Twist the swab over and sample the

same surface 10 more times to ensure this side of the swab has also collected biofilms. Avoid moving your hand onto the white applicator stick.

3. It is now OK to put the showerhead down into the tub or onto a counter.
4. Once you have collected the sample, twist and remove the cap from the sterile collection tube that remains in the swab package and CAREFULLY place the soiled swab into the transport tube, being careful not to touch the swab tips with your fingers.
5. Cap tightly. Label the tube with a Sharpie or pre-prepared label that contains at a minimum the following information - the date, your initials, sample ID, and sample type (*i.e.,* showerhead biofilm (SHB)).
6. Place labeled tubes in a ziploc bag and refrigerate swab until processing.

HOW TO SAMPLE A NON-REMOVABLE SHOWERHEAD:

Locate the showerhead in your home that is most often used. Swabbing should be done when then showerhead is as dry as possible — do not use a showerhead that has been recently used or is wet.

1. Locate the non-removeable showerhead. Take a representative picture using your cell phone or camera.
2. Carefully open a new swab package. To do so, carefully pry open the swab package. Remove the swab by grabbing the RED handle and avoid laying the swab down on non-sterile surfaces; keep swab sterile! **Be careful not to touch anywhere along the swab and only handle the swab by the red handle tip.**
3. Firmly and thoroughly, swab the **OUTSIDE SURFACE** of the showerhead (the side from which the water would emerge) using a firm back and forth as well as a circular motion, imagining that you are using the swab to clean the showerhead surface as completely as possible. Don't forget to get into the grooves on the showerhead face. Twist the swab over and sample the same surface 10 more times to ensure this side of the swab has also collected biofilms. Avoid moving your hand onto the white applicator stick.
4. Once you have collected the sample, twist and remove the cap from the sterile collection tube that remains in the swab package and CAREFULLY place the soiled swab into the transport tube, being careful not to touch the swab tips with your fingers.
5. Cap tightly. Label the tube with a Sharpie or pre-prepared label that contains at a minimum the following information - the date, your initials, sample ID, and sample type (*outside SHB*).
6. Place labeled tubes in a ziploc bag and refrigerate swab until processing.

ICE MACHINE SAMPLING

1. Locate the ice machine. Take a representative picture of the ice machine using your cell phone or camera. Be sure to capture both the ice and water spouts.
2. Obtain one of the sterile dual-tipped Flock swabs.
3. Carefully open a new swab package. To do so, carefully pry open the swab package. Remove the swab by grabbing the RED handle and avoid laying the swab down on non-sterile surfaces; keep swab sterile! **Be careful not to touch anywhere along the swab and only handle the swab by the red handle tip.**
4. Insert the swab up into the plastic ice dispensing "shoot" – highlighted in red in the image below.
5. Firmly and thoroughly, swab all sides of the plastic ice shoot using a "stirring" motion to move the swab in and around the opening - imagine that you are using the swab to methodically clean the surface. Rotate the swab as you swirl to ensure all portions of the swab has been exposed to biofilms. Rotate and swab 10 times. Avoid moving your hand onto the white applicator stick.

6. Once you have collected the sample, twist and remove the cap from the sterile collection tube that remains in the swab package and CAREFULLY place the soiled dust swab into the transport tube, being careful not to touch the swab tips with your fingers.
7. Cap tightly. Label the tube with a Sharpie or pre-prepared label that contains at a minimum the following information: the date, your initials, sample ID, and sample type (*i.e.,* ice machine).
8. Repeat steps 2-7 for the water spout on the ice machine.
9. Place labeled tubes in a ziploc bag and refrigerate swabs until processing.

DRINKING FOUNTAIN SAMPLING

Sampling should be done when the spigot is as dry as possible—avoid sampling a spigot if it has been recently used or is wet. If spigot is wet, DO NOT dry off. If possible, return to faucet at another time.

1. Locate the drinking fountain. Take a representative picture of the drinking fountain using your cell phone or camera.
2. Obtain one of the sterile dual-tipped Flock swab.
3. Carefully open a new swab package. To do so, carefully pry open the swab package. Remove the swab by grabbing the RED handle and avoid laying the swab down on non-sterile surfaces; keep swab sterile! **Be careful not to touch anywhere along the swab and only handle the swab by the red handle tip.**

4. *If mesh sampling* - Firmly and thoroughly swab the **point on the spigot where water exits** in a back and forth motion 10 times as well as a circular motion - imagine that you are using the swab to "clean" the surface as completely as possible. Twist the swab over and sample the same surface 10 more times to ensure this side of the swab has also collected biofilms. Avoid moving your hand onto the white applicator stick.
   OR
   If open pipe sampling – Insert the swab into the pipe and swab the sides of the pipe using a circular, firm stirring motion - similar to stirring your coffee – 10 times. Twist the swab over and sample the same surface 10 more times to ensure all sides of the swab has collected pipe biofilm. Avoid moving your hand onto the white applicator stick.
5. Once you have collected the sample, twist and remove the cap from the sterile collection tube that remains in the swab package and CAREFULLY place the soiled swab into the transport tube, being careful not to touch the swab tips with your fingers.
6. Cap tightly. Label the tube with a Sharpie or pre-prepared label that contains at a minimum the following information: the date, your initials, sample ID, and sample type (*i.e.*, left drinking fountain).
7. Place labeled tubes in a ziploc bag and refrigerate samples until processing.

WATER FEATURE SAMPLING

1. Locate the water feature. It may be a fountain, pool, or water wall. Take a representative picture of the water feature before sampling using your cell phone or camera.
2. Obtain one of the sterile dual-tipped Flock swabs.
3. Carefully open a new swab package. To do so, carefully pry open the swab package. Remove the swab by grabbing the RED handle and avoid laying the swab down on non-sterile surfaces; keep swab sterile! **Be careful not to touch anywhere along the swab and only handle the swab by the red handle tip.**
4. Sample the dry "scumline" (see image of a pool below) – located at the air-water interface. Rotate the swab around as you sample the dry area right above the water line (see image of a pool below) and ensure all portions of the swab has been exposed to the biofilm. Rotate and swab 10 times. Avoid moving your hand onto the white applicator stick.
5. Once you have collected the sample, twist and remove the cap from the sterile collection tube that remains in the swab package and CAREFULLY place the soiled swab into the transport tube, being careful not to touch the swab tips with your fingers.
6. Cap tightly. Label the tube with a Sharpie or pre-prepared label that contains at a minimum the following information: the date, your initials, sample ID, and sample type (*i.e.,* water feature).
7. Place labeled tubes in a ziploc bag and refrigerate swabs until processing.