Tongue dorsum swab processing for the detection of tuberculosis with Cepheid Xpert® MTB/RIF Ultra

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Abstract

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Tongue swabs have shown promise as alternatives to sputum for detecting *Mycobacterium tuberculosis* (MTB) in patients with pulmonary tuberculosis (TB). Some of the most promising results have come from studies that used manual quantitative PCR (qPCR) to analyze swabs. Studies using the automated Cepheid Xpert® MTB/RIF Ultra qPCR test (Xpert Ultra) to analyze tongue swabs have yielded more modest results. Using tongue swabs collected from TB-negative volunteers and spiked with cultured MTB cells, this work screened nearly 30 swabbing, storage, and extraction methods for using Xpert Ultra with tongue swabs. Three methods were then subjected to more rigorous evaluation. The most promising method, in which two COPAN FLOQswabs were collected and combined into a single tube and processed with Cepheid's Sample Reagent, yielded a 95% limit of detection (LoD) of 76.5 CFU/swab (95% CI 53.7 – 103.4), with an error rate of 4.6%. This method exhibited suitable sensitivity and low error rate, and is prioritized for clinical evaluations. In comparison, the clinically validated manual qPCR

method returned a LoD of 53.5 CFU/swab (95% CI 36.9 – 73.0), whereas Cepheid's experiments in sputum Xpert Ultra yielded a LoD of 15.6 CFU/mL sputum (95% CI 12.2 – 23.1).

Biology and Epidemiology of Tuberculosis

Species of the *Mycobacterium tuberculosis* complex (MTBC) are a genetically-similar group of bacteria capable of causing tuberculosis in humans and other animals. While most identified species in the MTBC are at least capable of infecting humans, the most prevalent strains infecting humans globally include *M. bovis* and *M. tuberculosis*, with *M. tuberculosis* responsible for the greatest proportion of tuberculosis (TB) disease (1, 2). Some strains, such as *M. africanum*, although rare globally, do represent another significant species in some locales (3). Members of the MTBC are nonmotile, aerobic, rod-shaped bacteria that have an outer coating of mycolic acid, which lend to MTBC members' heartiness and resistance to Gram staining techniques.

When *M. tuberculosis* or other species in the MTBC, contained in small aerosol droplets, are inhaled by a susceptible individual, viable bacilli may travel down to the alveoli of the lungs. Macrophages begin ingesting the invading bacilli and undergo phagocytosis. Some bacilli are able to evade phagocytosis by inhibiting macrophage phagolysosome development and thus survive within the macrophages. These processes induce a variety of immune pathways, resulting in the recruitment of many more immune cells. The immune cells surround the bacilli and infected macrophages forming a granuloma. Upon some type of immune system stressor, such as HIV, malnutrition, or genetic predisposition, the granuloma may burst, releasing viable bacilli (4–7). At this point an individual has developed active pulmonary TB. The individual is infectious in this stage of infection. Some peoples' immune systems may effectively eliminate the infection, others may go their entire lives only latently infected, whereas others develop

active TB, extrapulmonary TB, subclinical TB, or incipient TB (8, 9). TB, like many diseases, lies on a spectrum in terms of severity and infectiousness within individuals.

TB remains a leading cause of infectious disease morbidity and mortality globally, despite the availability of effective therapeutics for susceptible strains of the bacteria (10). It is estimated that approximately one-quarter of the world's population (1.7 billion) is infected with latent TB (11). There are an estimated 10 million incident active TB cases every year and over a million deaths per year despite the availability of treatment for most forms of the disease (10, 12). Despite progress on most disease measures (incidence, deaths, number on TB treatment), globally, we continue to fall behind on many of the goals that have been set by the UN high-level meeting on TB, the Sustainable Development Goals, and End TB Strategy goals (10).

The COVID-19 pandemic has potentially set back many of the gains that have been made in the fight against TB in recent years (13–15). In many countries during the early part of the pandemic, other infectious disease control programs, including HIV, TB, and Malaria programs had funds and resources diverted (15). This diversion of resources along with lockdown measures drastically decreased the number of TB diagnoses in a number of high-burden countries compared to their typical levels (13, 14). The interplay of lockdowns and other COVID-19 control measures on TB are yet to be fully understood, however the "WHO fears that over half a million more people may have died from TB, simply because they were unable to obtain a diagnosis." (16). Rebuilding and reallocating case finding infrastructure along with diagnostic innovation is greatly needed in order to limit the impacts of COVID-19 on the TB crisis.

Current Diagnostics and Issues

There are a number of barriers to TB control and prevention, including limited and sub-optimal diagnostics currently available (17).

Historically, TB diagnosis in resource-limited countries has relied heavily on passive case finding, in which people with active symptoms generally report to a healthcare facility. Active case finding is a much more expensive and labor-intensive effort. Active case finding efforts are most often limited to close contacts of persons with tuberculosis, in which these close contacts will be tested over the course of months and years (18, 19). Some active case finding efforts may focus on populations that are at higher risk of disease, such as those that are diagnosed with HIV (20, 21). Despite these targeted active case-finding efforts, many individuals remain undiagnosed. The World Health Organization estimates that some 2.9 million people with active TB went undiagnosed in 2019, often referred to as 'the missing millions' (10). Implementation of such case finding programs are often not standardized in part due to the resource intensity demanded. Another limiting factor in active case finding however is the difficulty in obtaining suitable diagnostic specimens.

Diagnosis of TB is generally based on clinical symptoms and/or with sputum-based diagnostics, including smear microscopy, sputum culture, and more recently and increasingly sputum-processed nucleic acid amplification tests (22). The production of sputum can be burdensome for the patient and hazardous for healthcare providers and others when proper aerosolization isolation precautions are not taken. In addition, sensitivity of sputum diagnostics are not ideal in all populations, including those with HIV co-infection (23). Children are also often unable to produce sputum on demand and frequently require burdensome sputum production procedures or collection of gastric aspirates (24).

While there have been recent progress in bringing TB diagnostics from the lab closer to the point of care, improving the throughput of systems, and improving immunologic diagnostics, limiting factors remain. The majority of microbiological diagnostics still rely on sputum, which can be difficult to collect and process. Different diagnostic samples and sample collection methods are needed to protect healthcare workers (HCWs) and other patients from exposure. Sputum production procedures induce coughing and are high risk when proper infection control procedures are not heeded (25, 26).

There is limited information on the incidence of HCW occupational TB infection and nosocomial/clinically-acquired incidence in patient populations. However, several retrospective studies and case reports highlight these hospital-based transmission events. During the peak of the HIV and AIDS epidemic in the US, there were many well-documented outbreaks of multidrug resistant TB (MDR TB) in healthcare settings in which other patients and HCWs became infected (27–29). A relatively recent study in South Africa looking at the incidence of MDR and extensively drug-resistant TB (XDR TB) hospitalizations of HCWs found that HCWs were over 5 and 6 times more likely to be hospitalized for MDR and XDR respectively than non-HCWs (30). This increased incidence of hospitalization is likely due to increased occupational exposures rather than other risk factors, and only moderately related to HCWs' increased tendency to seek medical attention. In an occupational outbreak investigation at a university hospital in Lima, Peru, looking at risk factors among medical staff diagnosed with active pulmonary tuberculosis during the study period, some of the risk factors included having contact with persons with active TB [OR = 9.62 (CI 4.25-23.52)] and having helped with collecting sputum [OR = 3.13 (CI 1.42-7.30)]. Lab staff were additionally at a much higher risk of disease than other medical workers and this was attributed to inadequate laboratory infrastructure (31).

In a quantitative risk analysis using animal-based (mouse infectivity study) and Wells-Riley (epidemiologic study) models, occupational TB infections in the U.S. were estimated to be 3288 and 6420 respectively; assuming that 2.5% of these infections progress to disease in their lifetime, that estimates that there are 82 and 161 cases of occupationally acquired TB disease per year in the U.S. depending on the model used (32).These documented cases, as well as the likely thousands of undocumented nosocomial and other occupational cases worldwide highlight the need for safer diagnostics for infection prevention and occupational safety.

Despite the increased risk and difficulty in collection, sputum remains the most common diagnostic specimen for MTB bacteriological confirmation.

Oral Swab Analysis (OSA) Development and Benefits

Various oral and oropharynx swabbing methods have been utilized in veterinary medicine for the detection of Mycobacterium spp. in a variety of animals including elephants, deer, and nonhuman primates among others (33–36). Based on these data, it was posited that similar swabbing methods could be utilized in humans under the assumption that MTB bacilli and/or DNA will deposit in sufficient quantities on the tongue dorsum. In these studies, after sample collection, swabs were stored in a Tris–EDTA-Sucrose-SDS lysis buffer (IHLB) and subsequently processed by boiling the sample, rendering the sample noninfectious. Samples were then extracted using a manual QIAGEN QIAamp DNA mini kit spin column protocol. Extracted DNA was mixed with master mix components utilizing a non-nested quantitative PCR (qPCR) targeting IS6110, an insertion sequence (mostly) specific to the MTBC (37, 38).

In such studies, sensitivities in adults relative to positive sputum Xpert or positive sputum culture have ranged from 88% to 93%, with specificities ranging from 79% to 92% (39–41).

Early studies utilized buccal swabs (40), however later studies found that tongue swabs were much more effective at picking up MTBC than buccal swabs (39).

Copan FLOQSwabs, are swabs with nylon fibers that are "flocked" referring to the orientation of the fibers, perpendicular to the shaft. This unique orientation of the fibers greatly increases the overall surface area, allowing for enhanced cell capture. Recent data by Wood and colleagues show that FLOQSwabs pick up approximately two times as much bacterial mass using a universal rDNA protocol as previously evaluated Puritan Purflock swabs (average ΔCq of 1.0; $p = 0.0064$; $n = 15$ of each swab type) (41).

This progression of sampling and methodologies have guided clinical evaluations as well as the present study to evaluate the specific combination of tongue swab samples collected using Copan FLOQSwabs. The combination of tongue swabbing with high-capture swabs ensures the best chance for MTBC capture and detection.

Oral swabs are a non-invasive sample specimen that can be easily and readily collected. As mentioned previously, sputum is difficult for many individuals to produce (especially on demand), whereas oral swabs can be collected by virtually anyone. Oral swabs may potentially be safer for healthcare workers and others as well since the collection process does not require an individual to cough, potentially aerosolizing infectious bacilli.

Automated Nucleic Acid Amplification Testing for TB

Automated nucleic acid amplification tests (NAATs) for MTB have been on the rise in the past decade and have dramatically improved the time to TB diagnosis in many settings. Automated NAAT systems were developed in order to eliminate many of the burdensome, resource intensive processes typically involved in manual NAATs. Nevertheless, the majority of these systems were still designed with sputum as the diagnostic specimen of choice. There are

now a number of automated NAATs for the diagnosis of TB including the Roche cobas® MTB testing cassettes for use on cobas® 6800/8800 Systems (42, 43), the WHO endorsed Eiken Chemical loop-mediated isothermal amplification TB-LAMP assay (44), the WHO endorsed Molbio Truenat™ MTB-RIF Dx chips for use on Truelab® PCR Analyzers (45, 46), and the WHO endorsed Cepheid Xpert® MTB/RIF (Xpert) and Xpert® MTB/RIF Ultra (Xpert Ultra). The Xpert and Xpert Ultra are currently the most widely used automated NAAT for the diagnosis of TB (10).

Cepheid Xpert® MTB/RIF and MTB/RIF Ultra

Xpert Ultra is a cartridge-based system in which a "lightly" processed sputum sample is pipetted into the sample portion of the cartridge and the cartridge is placed into one of the Gene Xpert module slots. The sputum sample processing prior to insertion into the cartridge involves adding two volumes of Cepheid's proprietary Sample Reagent (SR), a solution of sodium hydroxide, isopropyl alcohol, and other ingredients (47). The SR renders the sample non-infectious after a 15-minute incubation but does not lyse (break open) bacilli in the sputum sample, which is important for the rest of the sample processing. Once the treated sample is loaded into the cartridge and the run started, the cartridge uses a plunger system to draw the sample through a membrane filter, trapping un-lysed bacilli as well as other cells and large debris. The system then draws a wash buffer and washes the cells and filter, sending the liquid to a waste compartment. A final "liquid reagent" is also drawn and passed over the cells and membrane. Then the system sonicates what is on the membrane, releasing the trapped cells' genetic material. The genetic material in the liquid reagent is drawn and mixed over the "reaction beads" containing the PCR master mix. Sample is loaded into the reaction tube and PCR parameters are initiated to heat and cool as the sample is being analyzed, measuring the sample's

fluorescence and delivering cycle threshold (Ct) values based on when different probes fluoresce above a certain limit of detection (48, 49). The original Xpert detected the presence of MTB by using a hemi-nested PCR, targeting the rifampicin resistance determining region (RRDR) of the rpoB gene. Beacons targeting five specific regions of the RRDR determine the presence of MTB and subsequently evaluates the melting temperatures of each beacon to detect any mutations within those regions that may confer resistance (48) (probe A – probe E). This assay provided a major rollout of near-patient testing, however, sensitivity in patients who were smear-negative was subpar (60.6% in Xpert compared to 77.5% in Xpert Ultra) (50). In the Xpert Ultra, the rpoB probe sequences were slightly revised to increase sensitivity and decrease the occurrence of false-Rif-resistance (rpoB1 – rpoB4). A mixed IS1081 and IS6110 probe, using the same fluorescent label, was also included in the Xpert Ultra to better identify paucibacillary and extrapulmonary TB in which the bacterial load may be too low for detection of the rpoB probes, but sufficient for the IS1081/IS6110 as these are multicopy gene elements ('trace readout'), with copy number depending on the specific MTBC species and strain (51–54). While the trace detection can be valuable in detecting low bacillary loads in certain populations, it has also been reported that some individuals with a history of TB infection may have a false-trace detection, and specificity of Xpert Ultra has often been reported lower than that of Xpert (50, 55, 56).

In studies that have used alternative sample specimens in place of sputum for the diagnosis of TB with the Xpert and Xpert Ultra systems, results have been variable in part due to inconsistent sample processing methods that are not necessarily optimized for the sample type.

Studies using cerebrospinal fluid, pleural fluid, and lymph node aspirates for the diagnosis of extrapulmonary tuberculosis in adults often have varied processing protocols. In cerebrospinal fluid with Xpert Ultra, there was a reported 89.4% sensitivity compared to culture.

This is contrasted with the lower sensitivities seen with pleural fluid (75.0%) and lymph node aspirate (70%) (57). The interstudy variability between reported sensitivities was often substantial, which may be the result of many factors including the comparator diagnostics and sample processing steps used.

There are a number of studies that have evaluated stool as an alternative to sputum with Xpert and Xpert Ultra. These studies have seen great variability in the sample processing steps. In a review article looking at nine studies evaluating stool with Xpert in pediatric populations, pooled sensitivity was found to be 67% (95% CI 52-79%) and specificity of 99% (98-99%) (58). Sensitivity was found to be higher among those living with HIV than those that were not living with HIV. In one such study diagnosing TB in hospitalized HIV-infected children, stool Xpert was found to have comparable sensitivity to gastric aspirate Xpert (59). This non-invasive sampling method in a historically hard to diagnose population is encouraging, however the majority of methods still utilize equipment that may not be available in some settings (high-speed centrifuges) (60–63). Extraction methods and standardization remains needed for this promising sampling methodology. Novel "disposable Stool Processing Kits" are an encouraging tool to make stool a more accessible diagnostic specimen for TB diagnosis (64).

There have now been a couple of studies to evaluate oral swab methodologies with the Xpert Ultra system. These studies have so far yielded more modest results than manual qPCR methods, possibly reflecting a lack of optimization of swab methods for Xpert Ultra. In one study by Mesman et al., researchers evaluated the sensitivity and specificity of OSA (using Whatman Omniswab with buccal collection) with Xpert Ultra compared to MTB culture (65). Thirty-three culture positive patients in Lima Peru were divided into one of three experimental testing groups. Group 1 had a buccal swab taken placed into our previously used lysis buffer (40)

and subsequently processed with 2 volumes of SR. Group 2 had a buccal swab placed into PBS and similarly processed with 2 volumes of SR. Group 3 buccal collection occurred onto a Whatman FTA card (a paper card with SDS) and were treated with 1.5mL of SR. The group found an overall sensitivity of OSA with Xpert Ultra of 45% and a specificity of 100%. The sample sizes were too small to observe significant differences between the groups. While clearly suboptimal in sensitivity, this study confirmed that MTB can be detected in oral swabs using Xpert Ultra. This paper utilized older methods including buccal swab collection as opposed to tongue collection and utilized Whatman Omniswabs as opposed to the Copan FLOQSwab. Both of these changes have been shown to greatly increase the bacterial biomass collected (41). In another study by Lima et al. researchers tested OSA methods in a mass screening setting at a prison in Brazil (66). Study methods were not clearly detailed but highlight the need for standardized methods. The researchers found that the overall sensitivity among 128 sputum Xpert positive individuals was 51%. Sensitivity was better in those that had ‘high’ or ‘medium’ diagnoses with sputum Xpert Ultra at 82% (32/39) as compared to those that had ‘low’ or ‘very low’ diagnoses at just 38% (34/89). The specificity among 128 sputum Xpert negative individuals was 100%.

The suboptimal sensitivities and variability in currently utilized OSA methods with Xpert Ultra highlights the need for further optimization of storage and extraction conditions. Given the excellent sensitivity that is observed with sputum in Xpert Ultra, and separately the excellent sensitivity that is observed in manual qPCR OSA, we hypothesize that these two methods can be suitably combined. Xpert Ultra in conjunction with OSA has the possibility of making TB testing safer, faster, and more widespread. These improvements over current diagnostic specimens and

methodologies could open the door to more active case-finding efforts, which will ultimately result in getting more individuals treated and fewer transmission events.

Article Submission to Journal

(References in this section are specific to article submission: pages 15-42)

Tongue swab processing for the detection of tuberculosis with Cepheid Xpert® MTB/RIF Ultra

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Abstract

Tongue dorsum swabs have shown promise as alternatives to sputum for detecting *Mycobacterium tuberculosis* (MTB) in patients with pulmonary tuberculosis (TB). Some of the most encouraging results have come from studies that used manual quantitative PCR (qPCR) to analyze swabs. In contrast, studies using the automated Cepheid Xpert® MTB/RIF Ultra qPCR test (Xpert Ultra) to analyze tongue swabs have yielded less encouraging results, possibly because Xpert Ultra is optimized for testing sputum, not tongue swab samples. Using Copan FLOQSwab® tongue swabs collected from TB-negative volunteers and spiked with cultured MTB cells, this study evaluated strategies for improving the sensitivity of Xpert Ultra with tongue swabs. In Method 1, FLOQSwab samples were processed by boiling, incubation, and mixing. This method exhibited a 95% limit of detection (LoD) of 22.3 CFU/swab (95% CI 15.3 – 34.3) but suffered from a high over-pressurization error rate of 40.6%. In Method 2, FLOQSwab samples were processed with Cepheid Sample Reagent (SR) following protocols similar to those used for sputum. In Method 3, samples consisting of two FLOQSwabs were collected and combined into a single tube and processed as in Method 2. Methods 2 and 3 yielded LoDs of 101.7 CFU/swab (95% CI 64.5 – 144.0) and 76.5 CFU/swab (95% CI 54.2 – 104.1), respectively, and had acceptable over-pressurization error rates (<5%). Method 3 approached the analytical sensitivity of the clinically validated manual qPCR method, which had an LoD of 53.5 CFU/swab (95% CI 36.9 – 73.0). Method 3 is therefore recommended for clinical evaluations.

Introduction

Each year, there are 10 million incident cases of TB, and over a million are killed, despite the availability of therapeutics for most forms of the disease (1). A significant barrier to current TB control and prevention is the difficulty of rapidly screening large numbers of people for active disease. Historically, TB diagnosis has relied on passive case finding, in which people with symptoms self-report to a healthcare facility for further evaluation. Pulmonary TB diagnosis most often relies on microbiological or molecular analysis of sputa for the presence of MTB cells or DNA to confirm diagnosis (2). The production of sputum can be burdensome for those providing the specimen and can be hazardous to healthcare providers and others when proper isolation precautions are not taken (3, 4). Moreover, children and people living with HIV are often unable to produce sputum on demand (5, 6). These limitations have spurred efforts to identify alternative TB sample types that are easier, less invasive, and safer to collect.

For analysis of sputum, products such as Cepheid's GeneXpert MTB/RIF and MTB/RIF Ultra (Xpert Ultra) have brought molecular testing closer to patients in the last twenty years (7, 8). Sputum sample processing with Xpert Ultra involves the addition of Cepheid's proprietary Sample Reagent (SR). This reagent liquifies and decontaminates the sample but does not lyse bacilli in the sputum sample. Once the treated sample is loaded into the cartridge and the run started, the sample is drawn through a membrane filter, trapping un-lysed bacilli. After wash steps, a sonic horn lyses the bacilli trapped on the membrane and releases their DNA. The DNA is then subjected to a hemi-nested qPCR, targeting the rifampicin resistance determining region (RRDR) of the rpoB gene (9). In Xpert Ultra, in addition to the rpoB probes, a mixed IS1081 and IS6110 probe is

included to enhance detection of low bacillary load samples (10). In laboratory testing, in control sputum spiked with MTB H37Rv, the 1st generation Xpert had a 95% limit of detection of 112.6 CFU/mL sputum and the 2nd generation Xpert Ultra improved that to 15.6 CFU/mL sputum (10).

Tongue swab-based TB diagnosis, also known as oral swab analysis (OSA), has emerged as a potential alternative to sputum (11). We have optimized and clinically validated tongue swab analysis procedures for manual modified QIAGEN QIAamp DNA mini kit spin column protocols and IS6110-targeted qPCR (12–15). Using manual qPCR methods, sensitivities in adults relative to positive sputum Xpert or positive sputum culture have ranged from 88% to 93%, with specificities ranging from 79% to 92% (12–14). Recently, others have developed a novel SLIM assay using homobifunctional imidoesters to detect MTB (16). In a preliminary, pre-print report, the use of tongue swabs with the SLIM assay was found to perform well, with 65.6% of clinically diagnosed TB patients testing positive with this method as compared to 43.4% with sputum Xpert. The specificity of the SLIM assay was 86.1% as compared to 100% in sputum Xpert (17).

Compared to sputum collection, tongue swabbing is faster, easier, and safer. Because tongue swabs are easy to collect from any person in any setting, OSA may be especially useful for non-clinical and community-based screening. Given the broad acceptance of Xpert and Xpert Ultra by TB control programs around the world, it is important to harmonize new sampling methods with Xpert platforms. To date, only a few studies have evaluated OSA in conjunction with Xpert. Success has been limited, with reported sensitivities ranging from ~45% relative to sputum culture (18) to ~51% relative

to sputum-Xpert (19). Although these studies also utilized alternative swabbing and sample handling methods, the results underscore the need to optimize and standardize Xpert Ultra protocols for detecting MTB in oral swabs.

This study sought to identify improved methods and provide updated guidance and protocols for using Xpert Ultra to detect MTB in tongue swabs. Using healthy volunteer swabs spiked with serially diluted MTB, three candidate methods were evaluated to determine 95% LoDs. When these LoDs were compared to that of the clinically validated QIAGEN method, an optimal protocol was identified. This report thereby provides updated guidance and protocols for using Xpert Ultra in conjunction with OSA tongue swabs. The results open the door to clinical evaluations to assess the utility of tongue swabs in conjunction with Xpert Ultra for the diagnosis of TB in a variety of settings.

Materials & Methods

Study Population and Ethics. Procedures for collection of swab samples from human participants were approved of by the University of Washington Human Subjects Division (STUDY00001840). Participants were at least 18 years of age and recruited from the University of Washington, School of Public Health, Department of Environmental and Occupational Health Sciences. They were assumed to be TB-negative based on low risk of exposure and lack of symptoms. A total of 15 participants were enrolled and sampled. Participants were contacted for repeated sampling as needed. In order to protect the safety of participants and study personnel during the

SARS-CoV-2 pandemic, swabs were self-collected with study staff leaving the room while collection took place.

Sample Collection, Spiking, and Storage. Tongue swab samples to be analyzed by Xpert Ultra were collected using COPAN FLOQSwabs® Regular Flocked Swab with an 80 mm or 30 mm breakpoint (COPAN Diagnostics Inc.). Participants self-swabbed along the breadth of the mid tongue dorsum, firmly pressing and rolling the swab head for approximately 10 seconds. Swab heads were then immediately spiked with 10 µL of serially-diluted cultured MTB H37Ra stored in 1x phosphate buffered saline with 15% glycerol and 0.05% Tween® 80 (PBSGT), or with blank PBSGT. Swab heads were then broken off into 5 mL polypropylene transport tubes (Corning) containing 800 µL sterile 1x Tris-EDTA (TE) buffer (10 mM Tris-HCl containing 1 mM EDTA•Na_2, pH 8.0) (Corning) for Methods 1-3. In Method 1 (1 FLOQSwab, Boil) and Method 2 (1 FLOQSwab, SR), each sample tube contained just one swab head, whereas in Method 3 (2 FLOQSwabs, SR), each sample tube contained two consecutively collected swab heads inserted side-by-side. Samples were either frozen at -80 °C until the day of processing or were processed the same day of collection (comparison of these two conditions can be found in the Supplemental Material; no significant difference was observed with respect to sensitivity, although effects on over-pressurization error rate is significant for Method 1).

Tongue swab samples to be extracted using the QIAGEN QIAamp DNA mini kit spin column protocol and analyzed by qPCR were collected as described previously (13) except that tongue swabs were self-collected using COPAN FLOQSwabs as described above, rather than by study personnel using alternative swab products. Swab

heads were spiked with MTB H37Ra as above, then broken off into 2 mL Fisherbrand™ microcentrifuge tubes with screw caps (Thermo Fisher Scientific) containing 500µL sterile 1x TE buffer. In this method, each sample tube contains just one swab head. Samples were frozen at −80°C until the day of processing.

Saliva swab samples were collected using COPAN FLOQSwabs by resting the swab on the underside of the tongue and allowing it to soak up saliva for 10 seconds. These swabs were not spiked with MTB H37Ra and received blank PBSGT. After collection, they were placed into 5 mL polypropylene transport tubes containing 800 µL sterile 1x TE buffer. Samples were processed on the same day as collection.

Method 1: Single FLOQSwab, Boil. Samples were thawed or held at ambient temperature for a minimum of 30 minutes prior to processing. Samples were heated to 100 °C for 10 minutes using a dry block heater (Thermo Scientific™ Digital Dry Baths/Block Heater) with water added to the block wells. Samples were removed from the heat block and allowed to cool either on ice or at ambient temperature for 5 minutes (rationale and comparison of these two methods can be found in the Supplemental Material; no significant difference was observed between the two). After cooling, two volumes (1600 µL) of sterile 1x TE buffer were added to the samples, for a total sample volume of 2.4 mL. The samples were shaken on a lab vortexer (GENIE® SI-0236 Vortex-Genie 2 Mixer, 120V) on setting 10 for 10-15 seconds. Samples were then allowed to sit at ambient temperature for 5 minutes and then shaken for an additional 10-15 seconds. Samples sat at ambient temperature an additional 10 minutes. The entire recoverable sample volume (2.0-2.4 mL) was drawn up into a sterile transfer pipette (included in the Xpert Ultra kit) and dispensed into the sample reservoir of the

Xpert Ultra cartridge, which was then loaded into a GeneXpert module, and run initiated. Samples were recorded as positive for MTB if GeneXpert software returned any positive result, including “Trace” diagnoses, in which the IS1081/6110 probe was positive but the rpoB probes were not.

Methods 2 and 3: Single (Method 2) and Double FLOQSwab (Method 3), SR. Samples were held/thawed at ambient temperature for a minimum of 30 minutes prior to processing. Sample processing steps were carried out in a biosafety cabinet. Two volumes (1600 µL) of Cepheid Sample Reagent (SR) were added to the samples. Samples were shaken on a lab vortexer for 10-15 seconds and then allowed to sit for 5 minutes before being shaken for an additional 10-15 seconds. Samples sat for an additional 10 minutes before being drawn up in the sterile transfer pipette. Recoverable volume was at least 2 mL. The sample was dispensed into the sample reagent reservoir of the Xpert Ultra cartridge, and analysis proceeded as in Method 1. A video standard operating procedure of this method was created to assist researchers who may wish to use this method. A YouTube link can be found in the Supplemental Material.

Saliva Xpert Ultra. Saliva samples were processed in the same manner as in Method 1.

Manual Extraction and qPCR Analysis. DNA extraction and concentration were accomplished using the modified QIAGEN QIAamp DNA mini kit (#51306) spin column protocol and an ethanol precipitation, as previously described (12, 13). Prior to opening the samples, they were heated to 100 °C for 10 minutes in a heat block. After the addition of Buffer AL, proteinase K, and ethanol, 700 µL of sample was loaded onto the spin column, with the remainder stored at -80 °C as a reserve in case retesting the

sample was necessary. After wash steps and as before (13), the samples were eluted twice using 150 µL of Buffer AE for a final volume of 300 µL, all of which was concentrated by ethanol precipitation to maximize sensitivity. The resulting dried DNA pellet was resuspended in 5 µL Buffer AE.

Master mix consisted of 1× Luna Universal Probe qPCR Master Mix (New England BioLabs, Inc., Cat # M3004L), 0.45 µM forward primer, 1.35 µM reverse primer, 0.25 µM FAM/MGBNFQ probe, 2.375 µL H2O. Each reaction comprised of 20 µL master mix, which was added directly to the resuspended DNA pellet. After an additional 10-minute incubation, during which the samples were vortexed at medium speed to mix, the total 25 µL volume was transferred to the PCR plate. Quantitative PCR was performed on the Applied Biosystems StepOnePlus Real-Time PCR system using the following reaction protocol: initial incubation at 95 °C for 10 min and 45 cycles of 95 °C for 15 seconds (denaturation) and 60 °C for 1 minute (annealing/extension). The primers, targeting IS6110 are those designed and described previously (20, 21). Samples were recorded as positive for MTB if the Cq value was ≤ 38, as was used in previous clinical evaluation (13).

Table 1 summarizes the processing steps for Methods 1-3 and manual qPCR extraction.

Modelling and Statistical Analysis. RStudio (Version 1.4.1103, RStudio, PBC) was used to model LoD plots and for inferential statistics.

LoD plots were modelled using modified code developed by Weir et al (22). This modelling program used our experimental dose-response data to fit an exponential model and obtain Maximum Likelihood Estimation (MLE) parameters. Exponential

models passed a goodness of fit test based on deviances from the MLE using a Chi-Squared distribution with an alpha of 0.05. Models were subjected to a bootstrap routine over 10,000 iterations to determine confidence intervals. Modifications of the original code included changing ID50/LD50 references and calculations to 95% limit of detection (LoD) references and calculations. Sensitivity and LoDs are reported on a CFU per swab basis (which is the same as the CFU per sample amount for Methods 1, 2, and QIAGEN, but is half of the CFU per sample basis for Method 3 which includes 2 swabs).

Fisher's exact tests were used to compare the over-pressurization error rates and sensitivities at specific concentrations between methods. Tests were 2-tailed and an alpha of 0.05 was used to determine significance.

Results

Strategy. Method 1, which relied on boiling and did not use SR, was selected for this study because it resembled the first steps of our previous manual methods (12–15). In early testing, Method 1 demonstrated excellent sensitivity, but as reported below it exhibited a high proportion of over-pressurization errors. Over-pressurization errors (Error code #2008) can result from the Xpert Ultra cartridge membrane becoming blocked or clogged. Blockage of the membrane increases the system pressure and causes the assay to abort if it increases beyond protocol limits.

Methods 2 and 3, which used SR, were assessed because they more closely resemble sputum processing protocols currently recommended by Cepheid for Xpert Ultra. As reported below, the addition of SR dramatically reduced the over-pressurization error rate as compared to Method 1.

Method 3 tested the hypothesis that the processing steps applied to one FLOQSwab in Method 2 could be applied to two FLOQSwabs to improve the sensitivity of TB detection while retaining a low error rate. A recent study demonstrated that a single flocked swab collects ≤10% of the tongue dorsum biomass that is available for TB testing (14). Therefore, if two flocked swabs are collected in succession, suspended in buffer at the same volume as a single swab, and tested as a single sample, then the collection of MTB bacilli from TB patients may be doubled on average relative to single swabbing. In order to compare this approach to single swabbing, each of the two swabs was spiked with the same number of bacilli as a single swab, and results are reported as CFU/swab (although an eluted double-swab sample contains twice as many bacilli as an eluted single-swab sample).

Sensitivities of Methods. With a total of 38 non-error samples represented in the dose-response data, Method 1 (boiling) exhibited an LoD of 22.3 CFU/swab (95% CI 15.3 – 34.3). Method 2 (SR, single swab), with 69 non-error samples, had an LoD of 101.7 CFU/swab (95% CI 64.5 – 144.0). Method 3 (SR, two swabs), with 64 non-error samples, had an LoD of 76.5 CFU/swab (95% CI 54.2 – 104.1). By way of comparison, the clinically validated manual qPCR method, here with 40 spiked samples, yielded an LoD of 53.5 CFU/swab (95% CI 36.9 – 73.0). Figure 1 plots the LoD curves of each of the described methods. Dose-response data are shown in Table 2.

Specificities of Methods. Methods 1-3 had no false-positives among negative control tongue swab samples (n = 27). Additionally, among all positive, non-trace diagnoses, there were no false-rifampicin-resistance determinations (n = 61). In the

manual QIAGEN qPCR method, among 10 negative control samples, there was one false-positive sample.

Over-Pressurization Error Rates of Methods. In Method 1, of a total 69 samples run, there were 28 over-pressurization errors (40.6%). For Method 2, of 78 samples run, there were no over-pressurization errors (0.0%). For Method 3, among 72 samples run, there were 3 over-pressurization errors (4.2%). The qPCR method is not subject to this type of error which is specific to Xpert Ultra platforms. Table 3 summarizes the LoDs and error rates for each of the methods.

The over-pressurization error rate of Method 1 was significantly greater than those of Method 2 ($p < 0.0001$) and Method 3 ($p < 0.0001$). Method 2 and Method 3's error rates were not significantly different ($p = 0.1082$).

To better understand what may be responsible for over-pressurization errors, saliva swabs were processed by the same protocol used for tongue dorsum swabs in Method 1. This analysis asked whether the over-pressurization errors were due to sample components (biofilm collected from the tongue dorsum), or to the swabs themselves (for example, release of swab fiber materials during boiling in the presence of sample matrix). Of 10 saliva swabs tested, none caused over-pressurization errors as compared to 28/69 total over-pressurization errors in Method 1 over all phases of this study ($p = 0.0119$). This result suggests that biomass components of tongue swab samples, not the swabs themselves, contribute to the high error rate.

Efforts to overcome over-pressurization errors in Method 1 included the addition of ionic and non-ionic detergents, modified heating and cooling procedures, cell-straining efforts, and the addition of SR at varying concentrations. All such methods

failed to adequately mitigate error rates. The rationale, methods, and results of these additional methods are detailed in the Supplemental Material. While the exact reasons for the over-pressurization errors with Method 1 remain unclear, we observed that the sample was visibly quite turbid after boiling, cooling, and vortexing. It is possible that proteins denatured during heating may fold into insoluble structures upon cooling, or that poorly fragmented cellular debris clogs the membrane.

Discussion

We have demonstrated that the Xpert Ultra with modified protocols can analyze tongue swabs with comparable analytical sensitivity to clinically validated manual qPCR methods for detecting MTB DNA in tongue swabs.

Method 1, evaluating 1 boil-inactivated FLOQSwab, had improved sensitivity compared to the manual qPCR method but suffered from an unsatisfactory error rate due to over-pressurization. Further investigation of boiling, heating, and mechanical homogenization protocols may be warranted to determine if it is possible to overcome the error problems without sacrificing the sensitivity of the method.

Method 2, evaluating 1 SR-inactivated FLOQSwab, may have limited utility due to its reduced sensitivity relative to the manual qPCR method. In contrast, Method 3, evaluating 2 SR-inactivated FLOQSwabs, approached the sensitivity of the qPCR manual method while maintaining an acceptable over-pressurization error rate of <5%. Therefore Method 3 is the most promising tongue swab method for the detection of MTB with Xpert Ultra. However, the superior sensitivity of Method 1 (LoD 22.3 CFU/swab with just a single swab) indicates that there is room for improvement over

Method 3. SR was designed to liquify and decontaminate sputum, but it may be too harsh for tongue swab samples, which may have less buffering capacity than sputum. This could increase MTB lysis and allow target DNA to pass through the cartridge membrane resulting in decreased sensitivity. Perhaps a more robust buffer or medium would be better able to protect the MTB cells in tongue swabs from lysis by SR.

Our manual Qiagen/qPCR method, although researched clinically, had not previously had its LoD formally assessed. This analysis provides a relevant baseline with which to compare future tongue swab methods for the detection of MTB.

This study had several limitations. Study samples came from a small cohort of 15 individuals. It is possible that with greater donor variability, trends in sensitivity and error rates may differ. Study participants all lived in Seattle, Washington, USA at the time of sampling. Persons living in different geographic locations may have different oral microbial communities that may influence error rate and sensitivity. Moreover, all study participants were over the age of 18. Pediatric tuberculosis case-finding is a major opportunity with tongue swab diagnostics, but it remains unclear if or how variability in their oral microbiota may impact our methods. Additionally, our study relied on negative tongue swab samples spiked with cultured MTB, whereas the MTB found in clinical tongue swab samples may be in a different physiological state.

Despite these limitations, this study presents a systematic evaluation of methods for testing tongue swab samples using the GeneXpert Ultra. Method 3 exhibits acceptable sensitivity and error rates, and is recommended for future clinical evaluations along a spectrum of TB disease. With further refinement, methods may

become even more sensitive and the requirement for double-swabbing may be obviated.

Tongue swab sampling has the potential to open the door to expanded tuberculosis case-finding efforts. This non-invasive, fast, and safer diagnostic specimen and processing method may provide a useful new tool in the global fight against TB.

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Figure 1. Limit of detection plots with 95 and 99% confidence intervals for Methods 1-3 and manual qPCR method.

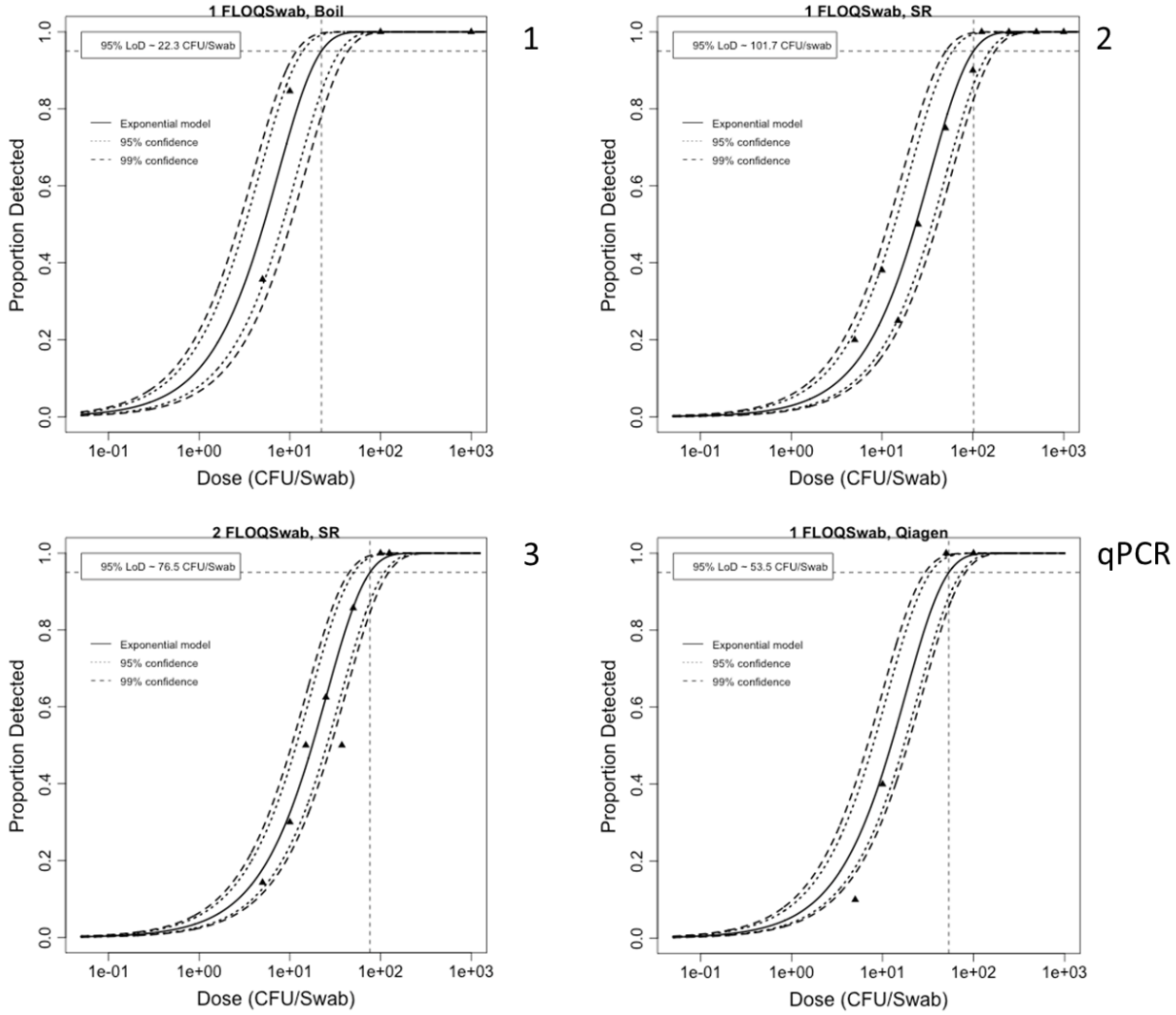

Table 1. Summary of Methods

Method 1	Method 2	Method 3	qPCR method
1 FLOQSwab. Boiled 10 minutes. Cooled 5 minutes. TE added. Incubate and mix. Load into Xpert.	1 FLOQSwab. SR added. Incubate and mix. Load into Xpert.	2 FLOQSwabs. SR added. Incubate and mix. Load into Xpert.	1 FLOQSwab. Boiled 10 minutes. QIAGEN extraction. Ethanol precipitation. Plate for qPCR.

Table 2. Dose response data used to plot the LoDs

	CFU/Swab										
	5	**10**	**15**	**25**	**37.5**	**50**	**100**	**125**	**250**	**500**	**1000**
Method 1	5/14*	11/13					8/8				3/3
Method 2	1/5	8/21	1/4	1/2		3/4	18/20	3/3	3/3	2/2	5/5
Method 3	1/7	3/10	4/9	6/9	4/5	6/7	10/10	7/7			
qPCR	1/10	4/10				10/10	10/10				

*****Number of samples with positive result/number of samples tested

Table 3. Comparison of Methods 1-3 and manual qPCR method limit of detections and over-pressurization error rate.

	Method 1	Method 2	Method 3*	qPCR method
LoD in CFU per swab (95% CI)	22.3 (15.3 - 34.3)	101.7 (64.5 - 144.0)	76.5 (54.2 – 104.1)	53.5 (36.9 – 73.0)
Over-Pressurization Rate % (N samples)	40.6% (69)	0.0% (78)	4.2% (72)	N/A

*Method 3 had two swabs per sample. LoD is CFU per swab as with the other methods.

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Article Submission to Journal Supplemental

Supplemental Materials

Method 1 Modified Conditions.

Additional Xpert Storage Buffers. Additional storage buffers that were evaluated included: 1x TE buffer supplemented with 0.1, 0.2, 0.3, or 0.5% TWEEN® 80 (Sigma-Aldrich) (v/v), or 1x TE buffer supplemented with 0.3, 0.5, or 3.0% sodium dodecyl sulfate (SDS) (Sigma-Aldrich) (w/w).

Cell Straining Xpert Ultra. These samples were stored in 1x TE buffer. Samples were processed in the same manner as in Method 1, except that prior to loading into the Xpert Ultra cartridge, the entire sample was added to a 10-micron syringe-driven cell strainer (pluriSelect) and slowly driven through the strainer into a collection tube. The flow-through was then added to the Xpert Ultra cartridge and run.

Boil with 2:1 and 1:1 SR Xpert Ultra. In both methods, samples were stored in 1x TE buffer. In both methods, samples were boiled for 10 minutes as was done in Method 1.

In the 2:1 method, after cooling at ambient temperature for 5 minutes, the sample received 2 volumes of SR (the same as in Methods 2 and 3) followed by the mixing and incubation durations as used in Methods 2 and 3. The entire sample was added to the Xpert Ultra cartridge.

In the 1:1 method, after cooling at ambient temperature for 5 minutes, the sample received the addition of 1200 µL of SR and 400 µL of 1x TE (such that TE and SR were in a 1:1 ratio). The mixing and incubation durations were the same as those used in Methods 2 and 3. The entire sample was added to the Xpert Ultra cartridge.

Heating or Boil Variations with Detergents. Samples in these methods were stored in one of the following conditions: 1x TE buffer supplemented with 0.1, 0.2, 0.3, or 0.5%

TWEEN 80 (v/v), or 1x TE buffer supplemented with 0.3, 0.5, or 3.0% SDS (w/w). Samples were processed in the same manner as in Method 1, or alternatively the heating temperature was lowered to 80 °C. A variation was that instead of adding 2 volumes of 1x TE, the samples receive 2 volumes of their respective storage buffer.

Results of other Xpert Ultra Methods. The aforementioned methods were subjected to screening criteria in order to determine which, if any modified methods could be better than Methods 1-3. For the preliminary screen, the following criteria were established that addressed both sensitivity and error rate goals. We sought methods that had fewer than 3 over-pressurization errors among 15 samples, at least 40% of samples correctly detected at 10 CFU/swab (among 5 non-errors), and at least 80% of samples correctly detected at 100 CFU/swab (among 5 non-errors).

Cell straining after boiling was hypothesized to reduce error rates as larger un-lysed cells and debris could be trapped on the strainer while the smaller MTB cells may be able to pass through the strainers. Clarity/turbidity of the sample was improved with 10-micron cell straining, and the strainers themselves had visible debris collected. Despite this, 4/6 (66.7%) samples still resulted in over-pressurization errors. This high error rate failed to meet our screening criteria for further consideration. Additionally, it indicated that the accumulation of small debris may be a significant issue. It was not warranted to use mesh sizes smaller than 10-micron as any smaller and we would expect to have trapped MTB (2-4 micron in length) on the strainer as well.

Given the failure to physically capture sufficient excess debris, chemical modifications to the full 2.4 mL of storage buffer and alterations to the heating temperature were explored as alternative methods to prevent the formation of insoluble

components. Tween® 80 (polysorbate 80), a nonionic detergent, and SDS, an ionic detergent, were supplemented into our TE storage buffer in increasing concentrations. The addition of Tween 80 was in an attempt to limit protein and lipid interactions that may have been resulting in coagulation. In similar but more intense fashion, addition of SDS may actively modify protein shape and cell structure. These altered storage buffers were evaluated using the standard boiling temperatures in Method 1 and also when heating to only 80 °C. It was hypothesized that lowering the heating temperature may result in reduced cell lysis and protein denaturing. Despite retaining excellent sensitivity comparable to Method 1, these methods were still unable to satisfactorily reduce the over-pressurization error rate (Supplemental Table 1).

Using SR (at full and diluted concentrations) after boiling was investigated as one additional effort that may eliminate the observed errors. In the 2:1 method that received 2 volumes of SR after boiling, the errors were eliminated, although unsurprisingly, the sensitivity became worse than that of SR alone (Method 2) with just 1/7 samples detected at 10 CFU/swab, falling below our sensitivity criteria. When using SR at a 1:1 ratio after boiling, the method reverted to having an excessive proportion of errors (4/6).

S. Table 1. Over-pressurization error rates of methods using TE supplemented with TWEEN® 80 or SDS, boiling or heating to 80 °C

Method	Over-Pressurization Errors (# Errors / Total Samples)	Method	Over-Pressurization Errors (# Errors / Total Samples)
Tween 80 0.1%; 80°C	3/8	**Tween 80 0.1%; Boil**	1/4
Tween 80 0.2%; 80°C	4/13	**Tween 80 0.2%; Boil**	0/2
Tween 80 0.3%; 80°C	2/6	**Tween 80 0.3%; Boil**	2/5
Tween 80 0.5%; 80°C	3/8		
SDS 0.3%; 80 °C	2/5	**SDS 0.3%; Boil**	2/4
		SDS 0.5%; Boil	3/4
		SDS 3.0%; Boil	2/2

Effect of freeze-thaw cycle on Method 1-3 sensitivity and over-pressurization error rate. Samples that could not be processed the same day that they were collected were frozen at -80 °C. Samples were thawed for 30 minutes prior to method processing.

For Method 1, there were 49 samples that underwent a freeze-thaw cycle with an over-pressurization error rate of 30.6%. There were 20 samples that were processed without the freeze-thaw cycle and there was an over-pressurization error rate of 65%. This difference was significant ($p = 0.0141$) and indicates that the freeze-thaw step may be effective in lowering the over-pressurization error rate of Method 1, although this is still well above the 5% error rate goal. Sample sizes for sensitivity comparisons of Method 1 were small, but at 10 CFU/swab, there were 5/7 detected in the freeze-thaw group as compared to 6/6 in the no freeze-thaw group ($p = 0.4615$).

There were no over-pressurization errors in all of Method 2. When comparing the sensitivity at 10 CFU/swab, there were 5/16 detected in the freeze-thaw group as compared to 3/5 detected in the no freeze-thaw group ($p = 0.3254$).

In Method 3, there were 32 samples that underwent a freeze-thaw cycle with an over-pressurization error rate of 0.0%. There were 40 samples that were processed without the freeze-thaw step with an over-pressurization error rate of 8.33%. Due to the low number of errors in the method overall, this did not rise to the level of significance ($p = 0.2562$), nevertheless the difference is interesting in that it matches the trend that was seen in Method 1. When comparing the sensitivity at 10 CFU/swab, there were 3/7 detected in the freeze-thaw group as compared to 0/3 in the no freeze-thaw group ($p = 0.475$).

Because differences in sensitivities were not statistically significant in any method, data from these conditions were pooled in the LoD assessment. The differences in error rate are interesting and worth pursuing further as a potential mechanism to reduce the over-pressurization error rate.

Method 1: Cooling on ice vs. ambient temperature after boiling. It was hypothesized that after boiling samples, the amount of particle formation and sedimentation may be controlled by cooling on ice rather than at ambient temperature. This comparison was undertaken in the hopes of reducing the proportion of over-pressurization errors that were observed with Method 1. Samples that were cooled at ambient temperature after boiling produced over-pressurization errors in 21/56 (37.5%) samples as compared to 7/13 (53.8%) of samples that were cooled on ice ($p = 0.3523$). Sample sizes too low to reliably measure differences in sensitivity, but there was not an

apparent difference given these limited sample sizes. 2/3 samples were detected at 10 CFU/swab and 3/3 detected at 100 CFU/swab when cooled on ice as compared to 9/10 samples detected at 10 CFU/swab and 5/5 detected at 100 CFU/swab when cooled at ambient temperature. Seeing as cooling on ice did not improve the proportion of errors nor clearly alter the method sensitivity, the majority of samples were cooled at ambient temperature. Because these differences were not significant, data from these two method variations were pooled for the dose-response data in Method 1 for modelling and calculation of the LoD.

YouTube Link of Method 2 & 3 Sample Processing

https://www.youtube.com/watch?v=5GDpuYLSKuI

Unsuccessful Methods and Suggestions for Method Improvement

In addition to the unsuccessful methods documented in the Supplemental Material of the journal submission, other unsuccessful methods included the evaluation of other swab types as well as the addition of lysozyme to samples.

Two additional swab types were briefly evaluated in addition to FLOQSwabs. The Halyard Dentaswab Oral Care Swab is a polyester foam swab with four rectangular foam surfaces. Participants swabbed ~10 times on each side of the swab when using these types of swabs. The swab was then spiked similar to the FLOQSwabs. Storage buffer and SR volumes were adjusted based on the size and adsorbent capacity of these swabs. In the case of SR treated Halyard swab samples, the sensitivity appeared lower than that of FLOQSwabs. Additionally, over-pressurization errors appeared with these swabs, potentially due to the increase in total biomass collected.

Another foam swab product, Toothette Oral Swabsticks, were also evaluated. These samples were collected in a similar manner to the Halyard swabs. However, the swabs were slightly smaller which allowed for the swab to be placed in a Salimetrics SalivaBio collection tube after sample inactivation. The swab could then be centrifuged in order to increase the volume recovery. However, this method was relatively cumbersome and would not be practical in many settings. Sensitivity also did not appear improved compared to FLOQSwabs. Foam swabs may not be ideal for MTBC detection using Xpert and Xpert Ultra as it may be that bacilli do not readily release from the swab or cannot otherwise be recovered from the sample.

The addition of lysozyme after boiling was evaluated as a potential method to reduce the over-pressurization errors. Lysozyme is a natural protein adept at attacking the cell wall of bacteria, making it a useful lysis agent against gram-positive bacteria. Unfortunately, despite the

expectation that it may reduce over-pressurization errors, this method caused all samples to fail either due to over-pressurization errors or due to a failure of the sample processing control (SPC). The SPC is a "spore cake" that contains non-infectious spores. This allows the Xpert to assure that MTB lysis has occurred, the specimen has been adequately processed, and inhibition of the PCR may be detected (77). While it isn't clear why the lysozyme would affect the SPC, given that this and over-pressurization errors occurred in the entirety of samples, this method of processing was abandoned.

As was discussed in the manuscript, different storage buffers may be able to improve upon the sensitivities of Methods 2 and 3 with the addition of SR. It is suspected that SR is too harsh of a reagent for our samples. TE buffer is often used as a storage buffer for nucleic acids, but other buffers may be more adept at keeping cells intact after contact with SR. Buffers without the addition of EDTA may in fact boost sensitivity as clumps of cells may be more likely to stay intact and therefore adhere to the Xpert cartridge membrane during the sample run.

A useful experiment would be to place MTB-spiked COPAN FLOQSwabs into MTB-negative sputum and treat the sample with SR. This experiment would help to identify whether the loss of sensitivity observed with the SR methods is related to the sample storage buffer or if it is an issue with releasing the bacilli from the swab head.

Boiling and heating inactivation of samples results in high precipitation of solids and therefore over-pressurization errors with Xpert Ultra. It remains unclear if these issues may be overcome using chemical or physical cleanup methods while maintaining the level of sensitivity seen with Method 1. The failure of cell-straining methods to adequately lower the over-pressurization error rate indicates that this issue may result from the accumulation of small debris

in addition to the larger debris and therefore may continue to pose issues if debris cannot be solubilized or even further fragmented.

Recommendations and Considerations for Clinical Evaluations

Method 3 (2 consecutively collected FLOQSwabs processed with SR) was the most promising method that we identified, balancing sensitivity with over-pressurization errors. This method is easily adaptable and transferable to settings that have Xpert platforms in place. No new reagents or equipment are necessary. Taken together, these factors make Method 3 a viable candidate for greater rollout if clinical evaluations are able to achieve sensitivities similar to that seen in the OSA manual qPCR methods.

Although we observed an unacceptably high error rate with Method 1 (1 FLOQSwab boiled) in our study population, it is unclear whether the prevalence of errors would be the same in other populations. Additionally, the sensitivity that was observed with Method 1 was still over three-fold better than that observed in Method 3. It may be worth exploring whether extra dilution steps may be able to alleviate the proportion of over-pressurization errors without sacrificing too much with respect to sensitivity.

OSA with Xpert Ultra cannot detect TB in all populations and scenarios. However, other non-invasive diagnostics may be able to supplement and boost the overall sensitivity. Cases missed by one test may be detected by the other and vice versa in certain scenarios. The urine lateral-flow lipoarabinomannan (LT-LAM) assay is an important and useful non-invasive diagnostic that has particular benefit in pediatric and HIV-positive populations (78, 79). Despite good diagnostic sensitivity in historically hard-to-diagnose populations, there are likely still more individuals that are missed if they were to only use the LT-LAM test. Having another non-invasive diagnostic such as OSA with Xpert Ultra may boost the sensitivity. This may still be a

cost-effective method of diagnosis in high-TB-burden settings in particular, and where diagnostic subsidy programs are in place (80, 81).

OSA with Xpert Ultra comparator methods should include sputum Xpert Ultra and at least initially with sputum culture for sensitivity and specificity determinations. Later evaluations may opt for using only one comparator method.

Conclusions

There remains a critical need for improved TB diagnostics. Making diagnostics faster, easier, and safer to collect with systems already widely available is an enticing and reachable goal. While room for optimization and refinement remain, the methods that we've identified and reported on warrant clinical evaluation and further development. We have shown that tongue swab samples can be quickly and easily processed in the laboratory setting for use with Xpert Ultra and that sensitivities approach those seen in manual qPCR methods.

When oral swabs are made compatible with Xpert Ultra, more work will need to be done to ensure that comprehensive systems are in place to train individuals, ensure funding of testing, ensure user acceptance, and that there is a sustainable linkage to care in place after a diagnosis. These were some of the limitations seen during the global rollout of the Gene Xpert and highlights that improvement of diagnostics are just one weapon in the fight against TB (82).

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Supplemental Material: Use of Laser Scanning Cytometry.

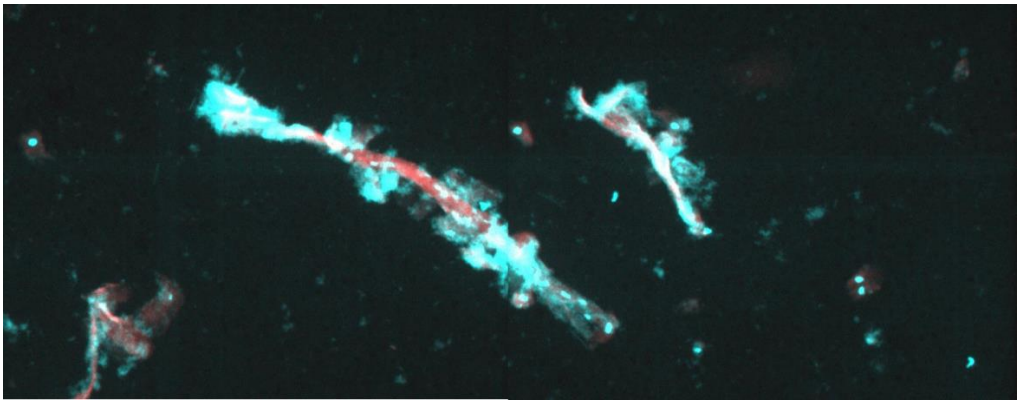
Because over pressurization errors with Xpert Ultra were a recurring issue in boiled samples, microscopic evaluation of cell lysate was briefly investigated in an attempt to better characterize the size and shape of cells and cellular debris.

Laser Scanning Cytometry (LSC) is a valuable tool allowing for the visualization and quantification of cell lysate by size and feature. This imaging was anticipated to allow for the prioritization of Xpert Ultra methods based on quantified debris captured by absorbance measurements.

Lysis methods evaluated by laser scanning cytometry (LSC) were only evaluated once and samples came from just a single donor in order to minimize sample variability.
Using a CompuCyte Corporation (Cambridge, Massachusetts, USA) iCys™ Imaging Cytometer (83) with 405 and 488nm laser diodes as well as blue, green, orange, and red photomultipliers, cell lysate from select lysis methods were stained with Hoechst dye at a concentration of 2μg/mL, placed on a slide, and subsequently imaged. Hoechst dye is a compound that complexes with adenine-thymine (A-T) rich regions of the DNA minor groove (84). Hoechst stain is membrane permeable by diffusion (85), making it a valuable dye to identify un-lysed cells in which the nuclear membrane is still intact. The dye is excited by ultraviolet light and fluoresces blue. Following staining and incubation, slides were imaged using LSC at 20x magnification and capturing a 500 x 387.8-micron composite image with individual fields of 62.5 x 48.475 microns and a resolution of 0.5 x 0.505 microns. Fluorescence was detected by the four photomultipliers. After images were captured, pictures were color-adjusted using the blue fluorescence to highlight DNA contrasted against green fluorescence, which captures other types of debris and materials that may auto fluoresce, such as swab fibers.

Ideal methods were expected to have debris patterns somewhere between the SR methods (Method 2) and the boil-only method (Method 1). Although Method 1 was seen to have high signal and visible debris, other boil methods supplemented with Tween80 and SDS showed only very little or very spotty signal/debris. Despite having little visual debris, these methods did not substantially reduce the over-pressurization error rate. LSC analysis of methods was therefore discontinued.

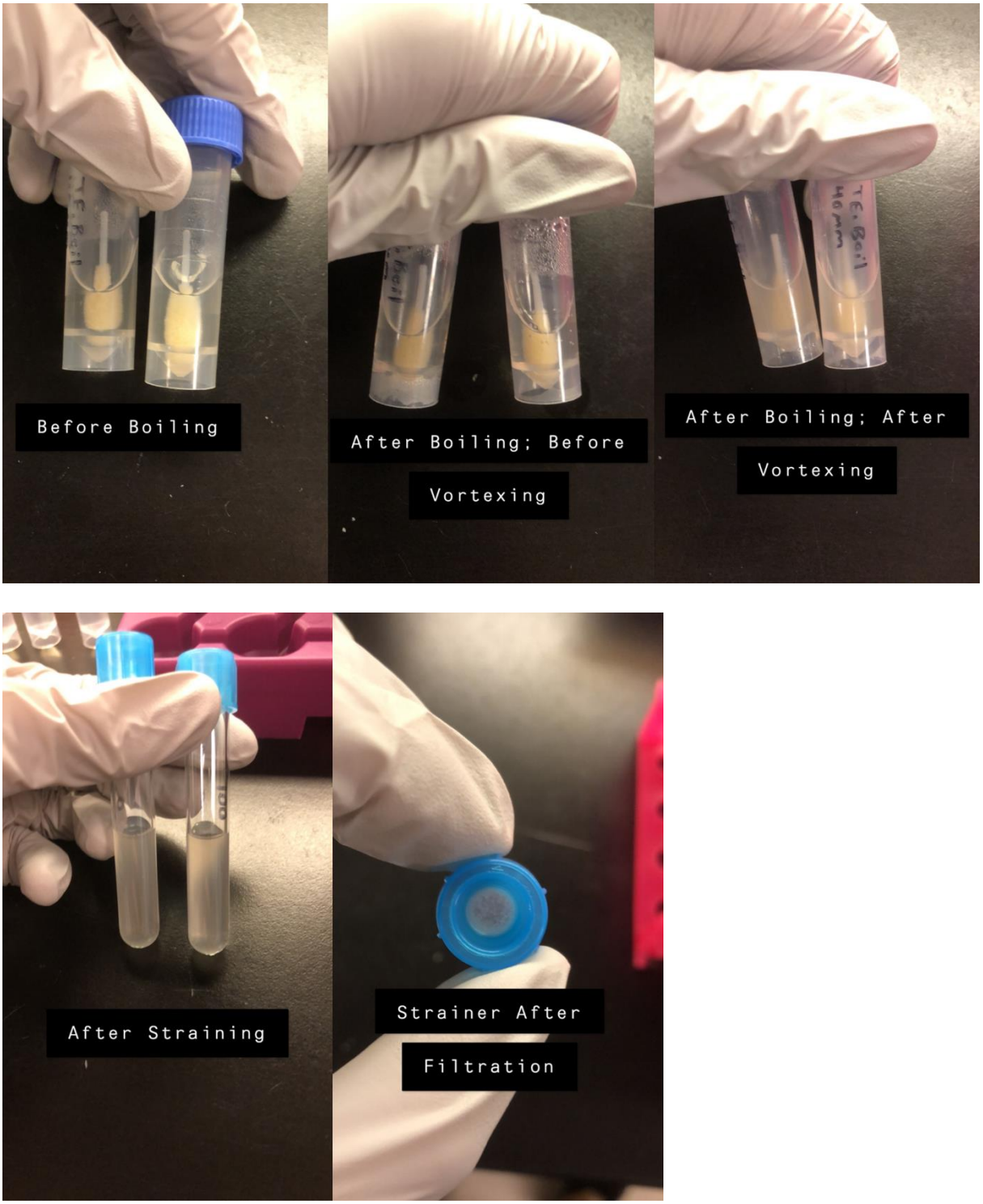
Supplemental Figure 1.



20x Magnification. 0.5 x 0.505 micron resolution. 125 x 48.475 microns. Method 1.

Despite our shortcomings, alternative methods of evaluating cell lysate may still be warranted. Methods that involved boiling were visibly quite turbid after heating and vortexting steps (see Supplemental Figure 2). This turbidity that is observed may be correlated with the proportion of over pressurization errors. Methods that result in less turbidity may therefore be prioritized for Xpert Ultra. That being said, despite cell straining methods moderately improving the clarity of the solution, these methods still resulted in a large proportion of errors as well.

Supplemental Figure 2.



Supplemental Material: Written Standard Operating Procedures.

UNIVERSITY OF WASHINGTON

STANDARD OPERATING PROCEDURE

Title: Sample Reagent processing of Copan FLOQSwabs® tongue swabs for analysis on Cepheid Xpert® MTB/RIF Ultra
Date of version: December 7, 2020
Number of pages: 2
Author: Grant R. Whitman
Revised by: Gerard A. Cangelosi
Reviewed by: Gerard A. Cangelosi

Introduction:
This protocol outlines the procedure for sample processing of single or double Copan FLOQSwabs® tongue swabs stored in 0.8mL of TE buffer using Cepheid's Sample Reagent (SR).
For information on how to properly collect and store tongue swab sample specimens, refer to the SOP, "Self-Collected Double Tongue Swabs for Analysis by GeneXpert MTB/RIF" by Rachel C. Wood. Note that the process is same for single- or double-swab samples.

Materials:

- Nitrile Gloves
- Lab Coat
- Class II Biosafety Cabinet (BSC)
- P1000 Pipette and Sterile Tips
- Sterile Transfer Pipette (provided in Ultra kit)
- Lab Vortex
- Timer
- Cepheid® Sample Reagent
- Xpert® MTB/RIF Ultra Cartridge

Safety Considerations:
Cepheid® Sample Reagent:
Hazard Statements:

- Flammable liquid and vapour
- Causes severe skin burns and eye damage.
- Causes serious eye damage
- Suspected of causing genetic defects.
- Suspected of damaging fertility or the unborn child.
- May cause damage to organs through prolonged or repeated exposure.

Refer to the SDS for more information: https://www.cepheid.com/en_US/safety-data-sheets

Biosafety:
Follow your institution's biosafety protocols for human samples. Samples are potentially infectious until sample inactivation has been achieved by sufficient contact time with SR.

Procedure:

1. Remove the sample from the freezer and place in the BSC. Allow the sample to thaw at ambient temperature at least half an hour until there is no ice remaining.
2. Pipet 2 volumes (1.6mL) of SR into the sample tube.
3. Ensure that the sample tube is sealed tightly and securely.
4. Vortex the sample tube in the BSC for 10-15 seconds.
5. Incubate for 5 minutes at ambient temperature.
6. Vortex the sample again in the BSC for an additional 10-15 seconds.
7. Incubate for an additional 10 minutes at ambient temperature.
8. Using a sterile transfer pipette, draw the entire sample volume avoiding production of bubbles as much as possible.
9. Expel the sample into the Ultra cartridge sample reservoir avoiding production of bubbles as much as possible. Ultra cartridge may be removed from the BSC and loaded into the GeneXpert module.
10. The sample tube should be discarded as biohazardous waste.

UNIVERSITY OF WASHINGTON

STANDARD OPERATING PROCEDURE

Title: Boil processing of Copan FLOQSwabs® tongue swabs for analysis on Cepheid Xpert® MTB/RIF Ultra
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Introduction:
This protocol outlines the procedure for sample processing of single or double Copan FLOQSwabs® tongue swabs stored in 0.8mL of TE buffer by boil inactivation.
For information on how to properly collect and store tongue swab sample specimens, refer to the SOP, "Self-Collected Double Tongue Swabs for Analysis by GeneXpert MTB/RIF" by Rachel C. Wood. Note that the process is same for single- or double-swab samples.

Materials:

- Nitrile Gloves
- Lab Coat
- Class II Biosafety Cabinet (BSC)
- P1000 Pipette and Sterile Tips
- Sterile Transfer Pipette (provided in Ultra kit)
- Heat Block or other Boiling Apparatus
- Water
- Lab Vortex
- Timer
- Sterile 1x TE Buffer
- Xpert® MTB/RIF Ultra Cartridge

Safety Considerations:
Biosafety:
Follow your institution's biosafety protocols for human samples. Samples are potentially infectious until inactivation has been achieved by boiling for a sufficient duration.

Procedure:
1. Remove the sample from the freezer and place in the BSC. Allow the sample to thaw at ambient temperature at least half an hour until there is no ice remaining.

2. While sample is thawing, set the heat block to a temperature >100°C. Add water to the heat block wells. Temperature should be confirmed by thermometer and visible boiling.
3. Once the sample has completely thawed and the water is at temperature, lower sample into the water carefully.
4. Heat sample for 10 minutes.
5. Remove sample using forceps or other PPE to avoid burns and transfer to BSC.
6. Sample is left to cool at ambient temperature for 5 minutes.
7. Pipet 2 volumes (1.6mL) of sterile 1x TE buffer into the sample tube.
8. Vortex the sample tube in the BSC for 10-15 seconds.
9. Incubate for 5 minutes at ambient temperature.
10. Vortex the sample again in the BSC for an additional 10-15 seconds.
11. Incubate for an additional 10 minutes at ambient temperature.
12. Using a sterile transfer pipette, draw the entire sample volume avoiding production of bubbles as much as possible.
13. Expel the sample into the Ultra cartridge sample reservoir avoiding production of bubbles as much as possible. Ultra cartridge may be removed from the BSC and loaded into the GeneXpert module.
14. The sample tube should be discarded as biohazardous waste.

Supplemental Material: Data Formatted by Date Run.

Key for Method Conditions:
FLOQSwab = Number of Copan FLOQSwabs per sample tube.
F-T = Sample was frozen and thawed before analysis.
Boil = Samples were boiled for 10 minutes as in Method 1.
80C = Samples were heated at 80°C for 10 minutes instead of boiled. All other steps carried out as in Method 1.
SR = Two volumes of Cepheid Sample Reagent were added, mixed, and incubated as in Method 2 unless other ratio is given (i.e. 1:2 means only one volume of SR was added to two volumes of sample storage buffer).
Lysozyme XminRT/37= 2.5mg lysozyme incubated in the sample tube after boiling and cooling for X minutes either at room temperature (RT) or 37°C (37).
TETx.x = TE buffer supplemented with x.x% of Tween80 (v/v).
TESDSx.x = TE buffer supplemented with x.x% of SDS (w/w).
IHLB = 'in-house-lysis-buffer' made up of 65mM Tris, 50mM EDTA, 50mM sucrose, 100mM NaCl, and 0.3% SDS.
Halyard Swab = Halyard Dentaswab Oral Care Swab. Participants swab ~10 times on each of the 4 sides of the swab.
Toothette = Toothette Oral Swabstick. Participants swab ~10 times on each of the 4 sides of the swab.
Salimetrics centrifugation = Swab and storage buffer were placed in a Salimetrics SalivaBio collection tube and centrifuged. Collected filtrate was put on Xpert Ultra.
Syringe-squeezed = swab heads were placed into a 30mL syringe and pressed in order to maximize recoverable volume.

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (C_T)	IS1081/6110 (C_T)	IS6110 (C_T)	Error Type
1	11.22.19	1 FLOQSwab, F-T, Boil	1	0	MTB Not Detected	N/A	N/A		
2	11.22.19	1 FLOQSwab, F-T, Boil	1	10	MTB Detected Very Low	28.8	22.1		
3	11.22.19	1 FLOQSwab, F-T, Boil	1	100	MTB Detected Very Low	28.7	20.6		
4	11.22.19	1 FLOQSwab, F-T, Boil	1	1000	MTB Detected Low	24.1	17.6		
5	11.22.19	1 FLOQSwab, F-T, Boil, SR	Other	10	MTB Not Detected	N/A	N/A		
6	11.22.19	1 FLOQSwab, F-T, Boil, SR	Other	100	MTB Detected Low	26.7	20.4		
7	11.22.19	1 FLOQSwab, F-T, Boil, SR	Other	1000	MTB Detected Low	26	18.9		
8	12.4.19	1 FLOQSwab, F-T, Boil	1	0	Error 2008				Overpressurization error
9	12.4.19	1 FLOQSwab, F-T, Boil	1	10	Error 2008				Overpressurization error
10	12.4.19	1 FLOQSwab, F-T, Boil	1	100	Error 2008				Overpressurization error
11	12.4.19	1 FLOQSwab, F-T, Boil	1	1000	Error 2008				Overpressurization error
12	12.4.19	1 FLOQSwab, F-T, Boil, SR	Other	0	MTB Not Detected	N/A	N/A		
13	12.4.19	1 FLOQSwab, F-T, Boil, SR	Other	10	MTB Not Detected	N/A	N/A		
14	12.4.19	1 FLOQSwab, F-T, Boil, SR	Other	100	MTB Detected Very Low	30.3	23.1		
15	12.4.19	1 FLOQSwab, F-T, Boil, SR	Other	1000	MTB Detected Low	26.2	19.9		
16	12.4.19	1 FLOQSwab, F-T, Boil	1	1000	MTB Detected Low	26.2	18.2		
17	12.4.19	1 FLOQSwab, F-T, Boil	1	10	MTB Detected Very Low	32.6	22.1		
18	12.4.19	1 FLOQSwab, F-T, Boil, SR	Other	10	MTB Detected Very Low	31.9	26.2		

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (C_T)	IS1081/6110 (C_T)	IS6110 (C_T)	Error Type
19	12.17.19	1 FLOQSwab, F-T, Boil	1	0	MTB Not Detected	N/A	N/A		
20	12.17.19	1 FLOQSwab, F-T, Boil	1	1000	MTB Detected Low	21.5	16.8		
21	12.17.19	1 FLOQSwab, F-T, Boil, SR	Other	100	MTB Detected Trace	N/A	28.1		
22	12.17.19	1 FLOQSwab, F-T, Boil, SR	Other	1000	MTB Detected Low	24.5	18.2		
23	12.17.19	1 FLOQSwab, Boil, SR	Other	0	MTB Not Detected	N/A	N/A		
24	12.17.19	1 FLOQSwab, Boil, SR	Other	1000	MTB Detected Low	25	18.5		
25	12.17.19	1 FLOQSwab, F-T, Boil, SR	Other	0	MTB Not Detected	N/A	N/A		
26	12.18.19	1 FLOQSwab, F-T, SR	2	0	Error 2005; Error 5007				Non-overpressurization error
27	12.18.19	1 FLOQSwab, F-T, SR	2	10	MTB Detected Trace	N/A	24.4		
28	12.18.19	1 FLOQSwab, F-T, SR	2	100	MTB Detected Very Low	28.3	21.8		
29	12.18.19	1 FLOQSwab, F-T, SR	2	1000	MTB Detected Low	23.8	17.9		
30	12.20.19	1 FLOQSwab, F-T, Boil	1	0	MTB Not Detected	N/A	N/A		
31	12.20.19	1 FLOQSwab, F-T, Boil	1	10	Error 2008				Overpressurization error
32	12.20.19	1 FLOQSwab, F-T, Boil	1	100	MTB Detected Very Low	28.3	20.3		
33	12.20.19	1 FLOQSwab, F-T, Boil	1	100	MTB Detected Low	26	19.2		
34	12.20.19	1 FLOQSwab, F-T, SR	2	0	MTB Not Detected	N/A	N/A		
35	12.20.19	1 FLOQSwab, F-T, SR	2	10	MTB Detected Trace	N/A	22.5		
36	12.20.19	1 FLOQSwab, F-T, SR	2	100	MTB Detected Low	27.3	22.6		
37	12.20.19	1 FLOQSwab, F-T, SR	2	1000	MTB Detected Very Low	29	20.5		
38	12.20.19	1 FLOQSwab, F-T, SR	2	0	MTB Not Detected	N/A	N/A		
39	12.20.19	1 FLOQSwab, F-T, SR	2	10	MTB Not Detected	N/A	N/A		
40	12.20.19	1 FLOQSwab, F-T, SR	2	100	Error 5007, 5017				Non-overpressurization error
41	12.20.19	1 FLOQSwab, F-T, SR	2	1000	MTB Detected Trace	N/A	21.8		
42	12.21.19	1 FLOQSwab, F-T, Boil	1	10	Error 2008				Overpressurization error
43	12.21.19	1 FLOQSwab, F-T, Boil	1	10	Error 2008				Overpressurization error
44	12.21.19	1 FLOQSwab, F-T, SR	2	0	MTB Not Detected	N/A	N/A		
45	12.21.19	1 FLOQSwab, F-T, Boil, SR	Other	0	MTB Not Detected	N/A	N/A		
46	12.21.19	1 FLOQSwab, F-T, Boil	1	10	MTB Detected Trace	N/A	25.4		
47	12.21.19	1 FLOQSwab, F-T, SR	2	100	MTB Not Detected	N/A	N/A		
48	12.30.19	1 FLOQSwab, F-T, Boil, 1:2SR	Other	10	Error 2008				Overpressurization error
49	12.30.19	1 FLOQSwab, F-T, Boil, 1:2SR	Other	10	Error 2008				Overpressurization error
50	12.30.19	1 FLOQSwab, F-T, SR	2	10	MTB Not Detected	N/A	N/A		
51	12.30.19	1 FLOQSwab, F-T, SR	2	10	MTB Not Detected	N/A	N/A		
52	1.2.20	1 FLOQSwab, F-T, Boil, 1:1SR	Other	10	MTB Not Detected	N/A	N/A		

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (C_T)	IS1081/6110 (C_T)	IS6110 (C_T)	Error Type
53	1.2.20	1 FLOQSwab, F-T, Boil, 1:1SR	Other	100	MTB Detected Trace	N/A	24.1		
54	1.2.20	1 FLOQSwab, F-T, SR	2	100	MTB Detected Trace	N/A	28.1		
55	1.2.20	1 FLOQSwab, F-T, SR	2	0	MTB Not Detected	N/A	N/A		
56	1.6.20	1 FLOQSwab, Boil, TET0.1	Other	100	MTB Detected Low	24.9	18.5		
57	1.6.20	1 FLOQSwab, Boil, TET0.1	Other	10	MTB Detected Low	36.6	22.6		
58	1.6.20	1 FLOQSwab, 80C, TET0.1	Other	100	Error 2008				Overpressurization error
59	1.6.20	1 FLOQSwab, 80C, TET0.1	Other	10	MTB Detected Low	31.9	20.9		
60	1.6.20	1 FLOQSwab, 80C, TET0.1	Other	100	MTB Detected Low	23.8	18.3		
61	1.6.20	1 FLOQSwab, 80C, TET0.1	Other	10	MTB Detected Trace	N/A	23.1		
62	1.6.20	1 FLOQSwab, 80C, TET0.1	Other	100	MTB Detected Low	26.9	18.9		
63	1.6.20	1 FLOQSwab, 80C, TET0.1	Other	10	MTB Not Detected	N/A	N/A		
64	1.7.20	1 FLOQSwab, F-T, Boil, TET0.1	Other	100	MTB Detected Trace	N/A	25.9		
65	1.7.20	1 FLOQSwab, F-T, Boil, TET0.1	Other	10	Error 2008				Overpressurization error
66	1.7.20	1 FLOQSwab, F-T, 80C, TET0.1	Other	100	Error 2008				Overpressurization error
67	1.7.20	1 FLOQSwab, F-T, 80C, TET0.1	Other	10	Error 2008				Overpressurization error
68	1.8.20	1 FLOQSwab, Boil, TET0.2	Other	100	MTB Detected Low	27.4	19.2		
69	1.8.20	1 FLOQSwab, Boil, TET0.2	Other	10	MTB Detected Very Low	33.9	21.9		
70	1.8.20	1 FLOQSwab, 80C, TET0.2	Other	100	MTB Detected Very Low	28.1	21.4		
71	1.8.20	1 FLOQSwab, 80C, TET0.2	Other	10	MTB Detected Very Low	30.8	24.9		
72	1.8.20	1 FLOQSwab, F-T, 80C, TET0.2	Other	100	Error 2008				Overpressurization error
73	1.8.20	1 FLOQSwab, F-T, 80C, TET0.2	Other	10	MTB Detected Trace	N/A	26.8		
74	1.8.20	1 FLOQSwab, Boil, Lysozyme 10minRT	Other	100	Error 2008				Overpressurization error
75	1.8.20	1 FLOQSwab, Boil, Lysozyme 10minRT	Other	100	Error 2008				Overpressurization error
76	1.8.20	1 FLOQSwab, Boil, Lysozyme 10minRT	Other	100	Invalid				
77	1.8.20	1 FLOQSwab, Boil, Lysozyme 10minRT	Other	10	Error 2008				Overpressurization error
78	1.9.20	1 FLOQSwab, F-T, Boil, Lysozyme 10min37	Other	10	Invalid				
79	1.9.20	1 FLOQSwab, F-T, Boil, Lysozyme 10min37	Other	10	Invalid				
80	1.9.20	1 FLOQSwab, F-T, Boil, Lysozyme 30min37	Other	100	Error 2008				Overpressurization error
81	1.9.20	1 FLOQSwab, F-T, Boil, Lysozyme 30min37	Other	10	Invalid				
82	1.10.20	1 FLOQSwab, Boil, Lysozyme 30min37, TET0.1	Other	100	Invalid				
83	1.10.20	1 FLOQSwab, Boil, Lysozyme 30min37, TET0.1	Other	10	Error 2008				Overpressurization error
84	1.10.20	1 FLOQSwab, SR	2	100	MTB Detected Trace	N/A	25.6		
85	1.10.20	1 FLOQSwab, SR	2	10	MTB Not Detected	N/A	N/A		
86	1.10.20	1 FLOQSwab, Boil, SR	Other	100	MTB Detected Trace	N/A	27.8		

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (C_T)	IS1081/6110 (C_T)	IS6110 (C_T)	Error Type
87	1.10.20	1 FLOQSwab, Boil, SR	Other	10	MTB Not Detected	N/A	N/A		
88	1.13.20	1 FLOQSwab, SR	2	100	MTB Detected Trace	N/A	25.2		
89	1.13.20	1 FLOQSwab, SR	2	10	MTB Detected Trace	N/A	26.2		
90	1.13.20	1 FLOQSwab, Boil, SR	Other	10	MTB Not Detected	N/A	N/A		
91	1.13.20	1 FLOQSwab, Boil, SR	Other	10	MTB Not Detected	N/A	N/A		
92	1.13.20	1 FLOQSwab, Boil, SR	Other	10	MTB Not Detected	N/A	N/A		
93	3.24.20	1 FLOQSwab, F-T, SR	2	0	MTB Not Detected	N/A	N/A		
94	3.24.20	1 FLOQSwab, F-T, SR	2	10	MTB Not Detected	N/A	N/A		
95	3.24.20	1 FLOQSwab, F-T, SR	2	100	MTB Detected Very Low	28.5	20.7		
96	3.24.20	1 FLOQSwab, F-T, SR	2	1000	MTB Detected Low	26.6	19.9		
97	3.26.20	1 FLOQSwab, F-T, SR	2	0	MTB Not Detected	N/A	N/A		
98	3.26.20	1 FLOQSwab, F-T, SR	2	10	MTB Not Detected	N/A	N/A		
99	3.26.20	1 FLOQSwab, F-T, SR	2	100	MTB Detected Trace	N/A	21.9		
100	3.26.20	1 FLOQSwab, F-T, SR	2	1000	MTB Detected Low	26.9	19		
101	6.23.20	1 FLOQSwab, F-T, SR	2	10	MTB Not Detected	N/A	N/A		
102	6.23.20	1 FLOQSwab, F-T, SR	2	100	MTB Not Detected	N/A	N/A		
103	6.23.20	1 FLOQSwab, F-T, SR, TET0.3	Other	0	MTB Not Detected	N/A	N/A		
104	6.23.20	1 FLOQSwab, F-T, SR, TET0.3	Other	100	MTB Detected Very Low	31.1	22.1		
105	6.24.20	1 FLOQSwab, F-T, SR	2	10	MTB Not Detected	N/A	N/A		
106	6.24.20	1 FLOQSwab, F-T, SR	2	100	MTB Detected Low	27.6	21.9		
107	6.24.20	1 FLOQSwab, F-T, SR, TET0.3	Other	0	MTB Not Detected	N/A	N/A		
108	6.24.20	1 FLOQSwab, F-T, SR, TET0.3	Other	100	MTB Detected Trace	N/A	25.5		
109	6.26.20	1 FLOQSwab, F-T, SR, TET0.3	Other	10	MTB Not Detected	N/A	N/A		
110	6.26.20	1 FLOQSwab, F-T, SR, TET0.3	Other	100	MTB Detected Very Low	28.7	20.5		
111	6.30.20	1 FLOQSwab, F-T, 80C, TET0.2	Other	10	Error 2008				Overpressurization error
112	6.30.20	1 FLOQSwab, F-T, 80C, TET0.2	Other	100	MTB Detected Very Low	34.4	22.2		
113	6.30.20	1 FLOQSwab, F-T, 80C, TET0.3	Other	10	Error 2008				Overpressurization error
114	7.1.20	1 FLOQSwab, F-T, 80C, TET0.2	Other	10	MTB Not Detected	N/A	N/A		
115	7.1.20	1 FLOQSwab, F-T, 80C, TET0.2	Other	100	MTB Detected Low	N/A	24.9		
116	7.7.20	1 FLOQSwab, F-T, 80C, TET0.2	Other	10	MTB Detected Trace	N/A	23.5		
117	7.7.20	1 FLOQSwab, F-T, 80C, TET0.2	Other	100	MTB Detected Low	24.5	18		
118	7.7.20	1 FLOQSwab, F-T, 80C, TET0.3	Other	10	MTB Detected Trace	N/A	26.4		
119	7.7.20	1 FLOQSwab, F-T, 80C, TET0.3	Other	100	MTB Detected Low	26	18.7		
120	7.8.20	1 FLOQSwab, F-T, 80C, TET0.2	Other	10	Error 2008				Overpressurization error

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (C_T)	IS1081/6110 (C_T)	IS6110 (C_T)	Error Type
121	7.8.20	1 FLOQSwab, F-T, 80C, TET0.2	Other	100	MTB Detected Very Low	29.3	22.7		
122	7.8.20	1 FLOQSwab, F-T, 80C, TET0.3	Other	10	MTB Not Detected	N/A	N/A		
123	7.8.20	1 FLOQSwab, F-T, 80C, TET0.3	Other	100	Error 2008				Overpressurization error
124	7.15.20	1 FLOQSwab, F-T, Boil, TET0.3	Other	10	MTB Detected Very Low	32.5	24.1		
125	7.15.20	1 FLOQSwab, F-T, Boil, TET0.3	Other	100	MTB Detected Low	27.8	20.2		
126	7.15.20	1 FLOQSwab, F-T, 80C, TET0.5	Other	10	MTB Detected Very Low	30.8	23.8		
127	7.15.20	1 FLOQSwab, F-T, 80C, TET0.5	Other	100	MTB Detected Low	27.2	19.3		
128	7.28.20	1 FLOQSwab, F-T, 80C, TET0.2	Other	100	Error 2008				Overpressurization error
129	8.7.20	1 FLOQSwab, F-T, 80C, TET0.5	Other	10	MTB Not Detected	N/A	N/A		
130	8.7.20	1 FLOQSwab, F-T, 80C, TET0.5	Other	100	Error 2008				Overpressurization error
131	8.7.20	1 FLOQSwab, F-T, 80C, TET0.5	Other	0	Error 2008				Overpressurization error
132	8.7.20	1 FLOQSwab, F-T, 80C, TET0.5	Other	10	MTB Detected Very Low	31.6	24.1		
133	8.10.20	1 FLOQSwab, F-T, Boil, TET0.3	Other	10	MTB Not Detected	N/A	N/A		
134	8.10.20	1 FLOQSwab, F-T, Boil, TET0.3	Other	100	Error 2008				Overpressurization error
135	8.10.20	1 FLOQSwab, F-T, Boil, TET0.3	Other	10	Error 5007				Non-overpressurization error
136	8.10.20	1 FLOQSwab, F-T, Boil, TET0.3	Other	100	Error 2008				Overpressurization error
137	8.11.20	1 FLOQSwab, F-T, 80C, TESDS0.3	Other	10	MTB Detected Very Low	32.7	23.5		
138	8.11.20	1 FLOQSwab, F-T, 80C, TESDS0.3	Other	100	MTB Detected Very Low	29.4	24.3		
139	8.11.20	1 FLOQSwab, F-T, 80C, TET0.5	Other	10	MTB Not Detected	N/A	N/A		
140	8.11.20	1 FLOQSwab, F-T, 80C, TET0.5	Other	100	Error 2008				Overpressurization error
141	8.12.20	1 FLOQSwab, F-T, 80C, IHLB	Other	10	Error 2008				Overpressurization error
142	8.12.20	1 FLOQSwab, F-T, 80C, IHLB	Other	100	Error 2008				Overpressurization error
143	8.12.20	1 FLOQSwab, F-T, 80C, IHLB	Other	10	MTB Detected Very Low	32.8	23.9		
144	8.12.20	1 FLOQSwab, F-T, 80C, IHLB	Other	100	Error 2008				Overpressurization error
145	8.13.20	1 FLOQSwab, F-T, Boil, TESDS0.3	Other	10	MTB Detected Very Low	30.8	24		
146	8.13.20	1 FLOQSwab, F-T, Boil, TESDS0.3	Other	100	Error 2008				Overpressurization error
147	8.13.20	1 FLOQSwab, F-T, Boil, TESDS0.3	Other	10	Error 2008				Overpressurization error
148	8.13.20	1 FLOQSwab, F-T, Boil, TESDS0.3	Other	100	MTB Detected Very Low	29.8	20.9		
149	8.14.20	1 FLOQSwab, F-T, 80C, TET0.3	Other	10	MTB Not Detected	N/A	N/A		
150	8.14.20	1 FLOQSwab, F-T, 80C, TESDS0.3	Other	0	Error 2008				Overpressurization error
151	8.14.20	1 FLOQSwab, F-T, 80C, TESDS0.3	Other	10	MTB Not Detected	N/A	N/A		
152	8.14.20	1 FLOQSwab, F-T, 80C, TESDS0.3	Other	100	Error 2008				Overpressurization error
153	8.25.20	1 FLOQSwab, F-T, Boil, 1:1SR	Other	10	Error 2008				Overpressurization error
154	8.25.20	1 FLOQSwab, F-T, Boil, 1:1SR	Other	100	Error 2008				Overpressurization error
155	8.25.20	1 FLOQSwab, F-T, Boil, 1:1SR	Other	10	Error 2008				Overpressurization error
156	8.25.20	1 FLOQSwab, F-T, Boil, 1:1SR	Other	100	Error 2008				Overpressurization error

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (CT)	IS1081/6110 (CT)	IS6110 (CT)	Error Type
157	8.26.20	1 FLOQSwab, F-T, Boil, TESDS0.5	Other	10	MTB Not Detected	N/A	N/A		
158	8.26.20	1 FLOQSwab, F-T, Boil, TESDS0.5	Other	100	Error 2008				Overpressurization error
159	8.26.20	1 FLOQSwab, F-T, Boil, TESDS0.5	Other	10	Error 2008				Overpressurization error
160	8.26.20	1 FLOQSwab, F-T, Boil, TESDS0.5	Other	100	Error 2008				Overpressurization error
161	8.31.20	1 Halyard Swab, Boil (3mL TE)	Other	100	Error 2008				Overpressurization error
162	9.1.20	1 Halyard Swab, SR (1mL TE, 2mL SR)	Other	100	MTB Not Detected	N/A	N/A		
163	9.2.20	1 FLOQSwab, F-T, Boil, TESDS3.0	Other	10	Error 2008				Overpressurization error
164	9.2.20	1 FLOQSwab, F-T, Boil, TESDS3.0	Other	100	Error 2008				Overpressurization error
165	9.3.20	1 FLOQSwab, F-T, SR	2	25	MTB Not Detected	N/A	N/A		
166	9.3.20	1 FLOQSwab, F-T, SR	2	50	MTB Not Detected	N/A	N/A		
167	9.4.20	1 FLOQSwab, F-T, SR	2	5	MTB Not Detected	N/A	N/A		
168	9.4.20	1 FLOQSwab, F-T, SR	2	15	MTB Not Detected	N/A	N/A		
169	9.4.20	1 FLOQSwab, F-T, SR	2	250	MTB Detected Low	26.4	19.5		
170	9.4.20	1 FLOQSwab, F-T, SR	2	500	MTB Detected Low	26.2	19.3		
171	9.4.20	1 FLOQSwab, F-T, Boil, 35um strain	Other	0	Error 2008				Overpressurization error
172	9.4.20	1 FLOQSwab, F-T, Boil, 35um strain	Other	100	Error 2008				Overpressurization error
173	9.9.20	1 FLOQSwab, F-T, SR	2	5	MTB Not Detected	N/A	N/A		
174	9.9.20	1 FLOQSwab, F-T, SR	2	15	MTB Detected Trace	N/A	24.8		
175	9.10.20	1 FLOQSwab, F-T, SR	2	250	MTB Detected Very Low	29.3	21.3		
176	9.10.20	1 FLOQSwab, F-T, SR	2	50	MTB Detected Trace	N/A	24.1		
177	9.11.20	1 FLOQSwab, F-T, Boil, 20um strain	Other	10	Error 2037				Non-overpressurization error
178	9.11.20	1 FLOQSwab, F-T, Boil, 20um strain	Other	100	Error 2008				Overpressurization error
179	9.11.20	1 FLOQSwab, F-T, Boil, 10um strain	Other	10	MTB Not Detected	N/A	N/A		
180	9.11.20	1 FLOQSwab, F-T, Boil, 10um strain	Other	100	Error 2008				Overpressurization error
181	9.14.20	1 Halyard Swab, F-T, increased V SR (2mL TE, 4mL SR)	Other	400	MTB Trace Detected	N/A	26.5		
182	9.14.20	1 Halyard Swab, F-T, increased V SR (2mL TE, 4mL SR)	Other	250	MTB Detected Very Low	30.7	25		
183	9.14.20	1 Halyard Swab, F-T, increased V SR (2mL TE, 4mL SR)	Other	250	MTB Not Detected	N/A	N/A		
184	9.14.20	1 Halyard Swab, F-T, increased V SR (2mL TE, 4mL SR)	Other	100	MTB Not Detected	N/A	N/A		
185	9.15.20	1 FLOQSwab, F-T, Boil, 10um strain	Other	10	Error 2008				Overpressurization error
186	9.15.20	1 FLOQSwab, F-T, Boil, 10um strain	Other	100	MTB Detected Very Low	34.9	22.6		
187	9.15.20	1 FLOQSwab, F-T, SR	2	125	MTB Detected Very Low	32.2	23.1		
188	9.15.20	1 FLOQSwab, F-T, SR	2	125	MTB Detected Very Low	30.6	22.6		
189	9.16.20	1 FLOQSwab, F-T, Boil, 10um strain	Other	10	Error 2008				Overpressurization error
190	9.16.20	1 FLOQSwab, F-T, Boil, 10um strain	Other	100	Error 2008				Overpressurization error
191	9.18.20	1 FLOQSwab, F-T, SR	2	5	MTB Not Detected	N/A	N/A		
192	9.18.20	1 FLOQSwab, F-T, SR	2	50	MTB Detected Very Low	31.6	24.8		

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (C_T)	IS1081/6110 (C_T)	IS6110 (C_T)	Error Type
193	9.18.20	1 FLOQSwab, F-T, SR	2	500	MTB Detected Low	25.7	18.8		
194	9.21.20	1 Toothette, SR, Salimetrics centrifugation	Other	400	MTB Detected Very Low	30.6	21.3		
195	9.21.20	1 Halyard Swab, SR (1mL TE, 2mL SR), Syringe-squeezed	Other	400	Error 2008				Overpressurization error
196	9.21.20	1 Halyard Swab, decreased V SR (0.8mL TE, 1.6mL SR), Syringe-squeezed	Other	400	MTB Detected Trace	N/A	24.3		
197	9.21.20	1 FLOQSwab, F-T, SR	2	5	MTB Not Detected	N/A	N/A		
198	9.21.20	1 FLOQSwab, F-T, SR	2	15	MTB Not Detected	N/A	N/A		
199	9.22.20	1 FLOQSwab, F-T, SR	2	50	MTB Detected Very Low	31.4	23.2		
200	9.22.20	1 FLOQSwab, F-T, SR	2	250	MTB Detected Very Low	29.4	19.9		
201	9.22.20	1 Toothette, SR, Salimetrics centrifugation	Other	40	MTB Not Detected	N/A	N/A		
202	9.23.20	1 FLOQSwab, F-T, SR	2	5	MTB Detected Very Low	30.7	24.1		
203	9.23.20	1 FLOQSwab, F-T, SR	2	15	MTB Not Detected	N/A	N/A		
204	9.23.20	1 Toothette, SR, Salimetrics centrifugation	Other	40	Error 2008				Overpressurization error
205	10.19.20	1 FLOQSwab, F-T, SR	2	100	MTB Detected Very Low	31.7	24		
206	10.19.20	2 FLOQSwab, F-T, SR	3	50	MTB Detected Trace	N/A	24.2		
207	10.19.20	2 FLOQSwab, F-T, SR	3	100	MTB Detected Trace	N/A	26.1		
208	10.20.20	1 FLOQSwab, F-T, SR	2	10	MTB Not Detected	N/A	N/A		
209	10.20.20	2 FLOQSwab, F-T, SR	3	5	MTB Not Detected	N/A	N/A		
210	10.20.20	2 FLOQSwab, F-T, SR	3	10	MTB Detected Trace	N/A	24.6		
211	10.20.20	1 FLOQSwab, F-T, SR	2	10	MTB Detected Trace	N/A	25.7		
212	10.20.20	2 FLOQSwab, F-T, SR	3	5	MTB Not Detected	N/A	N/A		
213	10.20.20	2 FLOQSwab, F-T, SR	3	10	MTB Not Detected	N/A	N/A		
214	10.21.20	1 FLOQSwab, F-T, SR	2	100	MTB Detected Very Low	29.2	23		
215	10.21.20	2 FLOQSwab, F-T, SR	3	50	MTB Detected Very Low	29.3	23.5		
216	10.21.20	2 FLOQSwab, F-T, SR	3	100	MTB Detected Very Low	31.3	21.4		
217	10.21.20	1 FLOQSwab, F-T, SR	2	10	MTB Not Detected	N/A	N/A		
218	10.21.20	2 FLOQSwab, F-T, SR	3	5	MTB Detected Trace	N/A	25.8		
219	10.21.20	2 FLOQSwab, F-T, SR	3	10	MTB Detected Trace	N/A	25.3		
220	10.22.20	1 FLOQSwab, F-T, SR	2	100	MTB Detected Trace	N/A	23.6		
221	10.22.20	1 FLOQSwab, F-T, SR	2	100	MTB Detected Trace	N/A	22.4		
222	10.22.20	2 FLOQSwab, F-T, SR	3	100	MTB Detected Very Low	38.8	23		
223	10.22.20	2 FLOQSwab, F-T, SR	3	100	MTB Detected Low	27.6	24.3		
224	10.23.20	1 FLOQSwab, F-T, SR	2	10	MTB Not Detected	N/A	N/A		

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (C_T)	IS1081/6110 (C_T)	IS6110 (C_T)	Error Type
225	10.23.20	1 FLOQSwab, F-T, SR	2	0	MTB Not Detected	N/A	N/A		
226	10.23.20	2 FLOQSwab, F-T, SR	3	10	MTB Not Detected	N/A	N/A		
227	10.26.20	1 FLOQSwab, F-T, SR	2	10	MTB Not Detected	N/A	N/A		
228	10.26.20	2 FLOQSwab, F-T, SR	3	0	MTB Not Detected	N/A	N/A		
229	10.26.20	2 FLOQSwab, F-T, SR	3	10	MTB Not Detected	N/A	N/A		
230	10.27.20	1 FLOQSwab, SR	2	100	MTB Detected Trace	N/A	23.2		
231	10.27.20	1 FLOQSwab, SR	2	0	MTB Not Detected	N/A	N/A		
232	10.27.20	2 FLOQSwab, SR	3	100	MTB Detected Low	27.7	21.5		
233	10.29.20	1 FLOQSwab, SR	2	10	MTB Detected Trace	N/A	24.4		
234	10.29.20	2 FLOQSwab, SR	3	10	MTB Not Detected	N/A	N/A		
235	10.29.20	2 FLOQSwab, SR	3	0	MTB Not Detected	N/A	N/A		
236	10.30.20	1 FLOQSwab, SR	2	100	MTB Detected Low	26.5	19.4		
237	10.30.20	2 FLOQSwab, SR	3	100	MTB Detected Very Low	28	22.8		
238	10.30.20	2 FLOQSwab, SR	3	0	MTB Not Detected	N/A	N/A		
239	11.2.20	1 FLOQSwab, F-T, SR	2	100	MTB Detected Trace	N/A	24		
240	11.2.20	1 FLOQSwab, F-T, SR	2	10	MTB Detected Trace	N/A	28.6		
241	11.2.20	2 FLOQSwab, F-T, SR	3	100	MTB Detected Trace	N/A	25.6		
242	11.2.20	2 FLOQSwab, F-T, SR	3	10	MTB Detected Trace	N/A	24.8		
243	11.3.20	1 FLOQSwab, SR	2	10	MTB Detected Trace	N/A	27.4		
244	11.3.20	2 FLOQSwab, SR	3	10	MTB Not Detected	N/A	N/A		
245	11.3.20	2 FLOQSwab, F-T, SR	3	100	MTB Detected Very Low	31.6	24.4		
246	11.4.20	1 FLOQSwab, SR	2	10	MTB Not Detected	N/A	N/A		
247	11.4.20	2 FLOQSwab, SR	3	10	MTB Not Detected	N/A	N/A		
248	11.4.20	1 FLOQSwab, F-T, SR	2	10	MTB Detected Low	27	18.7		
249	11.4.20	2 FLOQSwab, F-T, SR	3	10	MTB Not Detected	N/A	N/A		
250	11.6.20	1 FLOQSwab, F-T, SR	2	100	MTB Detected Very Low	28.1	20.2		
251	11.6.20	1 FLOQSwab, F-T, SR	2	0	MTB Not Detected	N/A	N/A		
252	11.12.20	1 FLOQSwab, SR	2	100	MTB Detected Low	27.4	20.5		
253	11.12.20	1 FLOQSwab, SR	2	100	MTB Detected Very Low	30.8	22.4		
254	11.12.20	2 FLOQSwab, SR	3	100	MTB Detected Very Low	30	21.9		
255	11.12.20	2 FLOQSwab, SR	3	100	MTB Detected Trace	N/A	24		
256	11.13.20	2 FLOQSwab, SR	3	25	MTB Detected Trace	N/A	22.6		
257	11.13.20	2 FLOQSwab, SR	3	125	MTB Detected Trace	N/A	23.7		

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (CT)	IS1081/6110 (CT)	IS6110 (CT)	Error Type
258	11.16.20	2 FLOQSwab, SR	3	25	MTB Detected Very Low	30.6	24.8		
259	11.16.20	2 FLOQSwab, SR	3	15	MTB Not Detected	N/A	N/A		
260	11.16.20	2 FLOQSwab, SR	3	25	MTB Detected Trace	N/A	25.3		
261	11.16.20	2 FLOQSwab, SR	3	15	MTB Detected Trace	N/A	24.7		
262	11.17.20	2 FLOQSwab, SR	3	25	MTB Not Detected	N/A	N/A		
263	11.17.20	2 FLOQSwab, SR	3	15	MTB Not Detected	N/A	N/A		
264	11.20.20	2 FLOQSwab, SR	3	5	Error 2008				Overpressurization error
265	11.20.20	2 FLOQSwab, SR	3	25	MTB Not Detected	N/A	N/A		
266	11.20.20	2 FLOQSwab, SR	3	5	MTB Not Detected	N/A	N/A		
267	11.20.20	2 FLOQSwab, SR	3	25	MTB Not Detected	N/A	N/A		
268	11.20.20	2 FLOQSwab, SR	3	0	MTB Not Detected	N/A	N/A		
269	11.20.20	2 FLOQSwab, SR	3	50	MTB Detected Very Low	36.6	23		
270	11.23.20	2 FLOQSwab, SR	3	5	MTB Not Detected	N/A	N/A		
271	11.23.20	2 FLOQSwab, SR	3	25	MTB Detected Trace	N/A	24.5		
272	11.23.20	2 FLOQSwab, SR	3	50	MTB Detected Trace	N/A	31.6		
273	11.23.20	2 FLOQSwab, SR	3	125	MTB Detected Low	26.9	21		
274	11.24.20	2 FLOQSwab, SR	3	5	Error 2008				Overpressurization error
275	11.24.20	2 FLOQSwab, SR	3	15	MTB Detected Trace	N/A	26		
276	11.24.20	2 FLOQSwab, SR	3	25	Error 2008				Overpressurization error
277	11.24.20	2 FLOQSwab, SR	3	50	MTB Detected Trace	N/A	24.4		
278	11.26.20	2 FLOQSwab, SR	3	37.5	MTB Not Detected	N/A	N/A		
279	11.26.20	1 FLOQSwab, SR	2	25	MTB Detected Trace	N/A	24.1		
280	11.26.20	1 FLOQSwab, SR	2	125	MTB Detected Trace	N/A	25.2		
281	12.2.20	2 FLOQSwab, SR	3	15	MTB Detected Very Low	33.1	21.4		
282	12.2.20	2 FLOQSwab, SR	3	37.5	MTB Detected Low	26.5	21.6		
283	12.2.20	2 FLOQSwab, SR	3	50	MTB Detected Trace	N/A	32.4		
284	12.2.20	2 FLOQSwab, SR	3	125	MTB Detected Very Low	31	20.4		
285	12.3.20	2 FLOQSwab, SR	3	0	MTB Not Detected	N/A	N/A		
286	12.3.20	2 FLOQSwab, SR	3	5	MTB Not Detected	N/A	N/A		
287	12.3.20	2 FLOQSwab, SR	3	15	MTB Detected Trace	N/A	26		
288	12.3.20	2 FLOQSwab, SR	3	125	MTB Detected Very Low	28.8	21.9		
289	12.4.20	2 FLOQSwab, F-T, SR	3	5	MTB Not Detected	N/A	N/A		
290	12.4.20	2 FLOQSwab, F-T, SR	3	15	MTB Not Detected	N/A	N/A		

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (CT)	IS1081/6110 (CT)	IS6110 (CT)	Error Type
291	12.4.20	2 FLOQSwab, F-T, SR	3	25	MTB Detected Low	27	23		
292	12.4.20	2 FLOQSwab, F-T, SR	3	125	MTB Detected Very Low	28.9	20.9		
293	12.7.20	2 FLOQSwab, F-T, SR	3	15	MTB Not Detected	N/A	N/A		
294	12.7.20	2 FLOQSwab, F-T, SR	3	37.5	Error 2005				Non-overpressurization error
295	12.7.20	2 FLOQSwab, F-T, SR	3	50	MTB Not Detected	N/A	N/A		
296	12.7.20	2 FLOQSwab, F-T, SR	3	125	MTB Detected Low	27.2	20.6		
297	12.11.20	2 FLOQSwab, F-T, SR	3	25	MTB Detected Very Low	29.3	24.3		
298	12.11.20	2 FLOQSwab, F-T, SR	3	37.5	MTB Detected Trace	N/A	25.3		
299	12.11.20	1 FLOQSwab (not swabbed), Boil	Air	0	MTB Not Detected	N/A	N/A		
300	12.16.20	2 FLOQSwab, F-T, SR	3	15	MTB Not Detected	N/A	N/A		
301	12.16.20	2 FLOQSwab, F-T, SR	3	37.5	MTB Detected Trace	N/A	24.1		
302	12.16.20	2 FLOQSwab, F-T, SR	3	125	MTB Detected Trace	N/A	25.9		
303	12.16.20	2 FLOQSwab, F-T, SR	3	37.5	MTB Detected Low	27.1	22.3		
304	12.16.20	1 FLOQSwab, Boil	1	10	Error 2008				Overpressurization error
305	12.16.20	1 FLOQSwab, Boil, Cooled on ice	1	10	Error 2008				Overpressurization error
306	12.16.20	1 FLOQSwab (not swabbed), Boil	Air	0	MTB Not Detected	N/A	N/A		
307	12.18.20	1 FLOQSwab, F-T, Boil	1	10	Error 2008				Overpressurization error
308	12.18.20	1 FLOQSwab, F-T, Boil	1	100	MTB Detected Low	26.3	20.3		
309	12.18.20	1 FLOQSwab, F-T, Boil	1	10	Error 2008				Overpressurization error
310	12.18.20	1 FLOQSwab, F-T, Boil	1	100	Error 2008				Overpressurization error
311	12.18.20	1 FLOQSwab, F-T, Boil, Cooled on ice	1	10	Error 2008				Overpressurization error
312	12.18.20	1 FLOQSwab, F-T, Boil, Cooled on ice	1	100	MTB Detected Very Low	29.9	21.7		
313	12.18.20	1 FLOQSwab, F-T, Boil, Cooled on ice	1	10	MTB Detected Trace	N/A	28.3		
314	12.18.20	1 FLOQSwab, F-T, Boil, Cooled on ice	1	100	MTB Detected Very Low	29.6	23.3		
315	12.21.20	1 FLOQSwab, Boil	1	10	Error 2008				Overpressurization error
316	12.21.20	1 FLOQSwab, Boil	1	100	Error 2008				Overpressurization error
317	12.21.20	1 FLOQSwab, Boil, Cooled on ice	1	10	Error 2008				Overpressurization error
318	12.21.20	1 FLOQSwab, Boil, Cooled on ice	1	100	Error 2008				Overpressurization error
319	12.23.20	1 FLOQSwab (not swabbed), Boil	Air	0	MTB Not Detected	N/A	N/A		
320	12.23.20	1 FLOQSwab (saliva), Boil	Saliva	0	MTB Not Detected	N/A	N/A		
321	12.23.20	1 FLOQSwab (not swabbed), Boil, Cooled on ice	Air	0	MTB Not Detected	N/A	N/A		
322	12.23.20	1 FLOQSwab (saliva), Boil, Cooled on ice	Saliva	0	MTB Not Detected	N/A	N/A		
323	12.24.20	1 FLOQSwab, F-T, Boil	1	10	MTB Not Detected	N/A	N/A		
324	12.24.20	1 FLOQSwab, F-T, Boil	1	100	Error 2008				Overpressurization error
325	12.24.20	1 FLOQSwab, F-T, Boil, Cooled on ice	1	10	MTB Not Detected	N/A	N/A		

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (C_T)	IS1081/6110 (C_T)	IS6110 (C_T)	Error Type
326	12.24.20	1 FLOQSwab, F-T, Boil, Cooled on ice	1	100	MTB Detected Very Low	28.5	22.6		
327	12.28.20	1 FLOQSwab (saliva), Boil	Saliva	0	MTB Not Detected	N/A	N/A		
328	12.28.20	1 FLOQSwab (saliva), Boil, Cooled on ice	Saliva	0	MTB Not Detected	N/A	N/A		
329	12.29.20	1 FLOQSwab, Boil	1	10	MTB Detected Trace	N/A	27		
330	12.29.20	1 FLOQSwab, Boil	1	100	Error 2008				Overpressurization error
331	12.29.20	1 FLOQSwab, Boil, Cooled on ice	1	10	Error 2008				Overpressurization error
332	12.29.20	1 FLOQSwab, Boil, Cooled on ice	1	100	Error 2008				Overpressurization error
333	12.30.20	1 FLOQSwab, Boil	1	10	MTB Detected Trace	N/A	24.2		
334	12.30.20	1 FLOQSwab, Boil	1	100	Error 2008				Overpressurization error
335	12.30.20	1 FLOQSwab (saliva), Boil	Saliva	0	MTB Not Detected	N/A	N/A		
336	12.30.20	1 FLOQSwab, Boil, Cooled on ice	1	10	MTB Detected Low	27.1	22.2		
337	12.30.20	1 FLOQSwab, Boil, Cooled on ice	1	100	Error 2008				Overpressurization error
338	12.30.20	1 FLOQSwab (saliva), Boil, Cooled on ice	Saliva	0	MTB Not Detected	N/A	N/A		
339	12.31.20	1 FLOQSwab, Boil	1	10	MTB Detected Trace	N/A	32.6		
340	12.31.20	1 FLOQSwab, Boil	1	100	MTB Detected Very Low	28.8	21.6		
341	12.31.20	1 FLOQSwab, Boil	1	10	MTB Detected Trace	N/A	25		
342	12.31.20	1 FLOQSwab, Boil	1	100	Error 2008				Overpressurization error
343	1.4.21	1 FLOQSwab (saliva), Boil	Saliva	0	MTB Not Detected	N/A	N/A		
344	1.4.21	1 FLOQSwab (saliva), Boil	Saliva	0	MTB Not Detected	N/A	N/A		
345	1.5.21	1 FLOQSwab (saliva), Boil	Saliva	0	MTB Not Detected	N/A	N/A		
346	1.5.21	1 FLOQSwab (saliva), Boil	Saliva	0	MTB Not Detected	N/A	N/A		
347	1.5.21	1 FLOQSwab, Boil	1	10	MTB Detected Trace	N/A	27.7		
348	1.5.21	1 FLOQSwab, Boil	1	10	Error 2008				Overpressurization error
349	1.6.21	1 FLOQSwab, F-T, Boil	1	10	MTB Detected Trace	N/A	26.2		
350	1.6.21	1 FLOQSwab, F-T, Boil	1	10	Error 2008				Overpressurization error
351	1.7.21	1 FLOQSwab, F-T, Boil	1	5	MTB Not Detected	N/A	N/A		
352	1.7.21	1 FLOQSwab, F-T, Boil	1	5	MTB Detected Trace	N/A	24.6		
353	1.7.21	1 FLOQSwab, F-T, Boil	1	5	MTB Not Detected	N/A	N/A		
354	1.7.21	1 FLOQSwab, F-T, Boil	1	5	MTB Detected Trace	N/A	24.7		
355	1.8.21	1 FLOQSwab, F-T, Boil	1	5	Error 2008				Overpressurization error
356	1.8.21	1 FLOQSwab, F-T, Boil	1	5	MTB Not Detected	N/A	N/A		
357	1.8.21	1 FLOQSwab, F-T, Boil	1	5	MTB Not Detected	N/A	N/A		
358	1.8.21	1 FLOQSwab, F-T, Boil	1	5	MTB Detected Very Low	33.8	23.5		
359	1.11.21	1 FLOQSwab, F-T, Boil	1	5	MTB Detected Very Low	30.8	24.2		
360	1.11.21	1 FLOQSwab, F-T, Boil	1	5	MTB Not Detected	N/A	N/A		

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (C_T)	IS1081/6110 (C_T)	IS6110 (C_T)	Error Type
361	1.11.21	1 FLOQSwab, F-T, Boil	1	5	MTB Detected Trace	N/A	26		
362	1.11.21	1 FLOQSwab, F-T, Boil	1	5	MTB Not Detected	N/A	N/A		
363	1.12.21	1 FLOQSwab, F-T, Boil	1	5	Error 2008				Overpressurization error
364	1.12.21	1 FLOQSwab, F-T, Boil	1	5	MTB Not Detected	N/A	N/A		
365	1.12.21	1 FLOQSwab, F-T, Boil	1	5	MTB Not Detected	N/A	N/A		
366	1.12.21	1 FLOQSwab, F-T, Boil	1	5	MTB Not Detected	N/A	N/A		
367	2.21.21	Qiagen Extraction	qPCR	100	MTB Detected			30.9926796	
368	2.21.21	Qiagen Extraction	qPCR	5	MTB Not Detected			Undetermined	
369	2.21.21	Qiagen Extraction	qPCR	10	MTB Not Detected			Undetermined	
370	2.21.21	Qiagen Extraction	qPCR	0	MTB Not Detected			Undetermined	
371	2.21.21	Qiagen Extraction	qPCR	0	MTB Not Detected			Undetermined	
372	2.21.21	Qiagen Extraction	qPCR	100	MTB Detected			30.90965462	
373	2.21.21	Qiagen Extraction	qPCR	100	MTB Detected			30.36872673	
374	2.21.21	Qiagen Extraction	qPCR	10	MTB Detected			37.10344315	
375	2.21.21	Qiagen Extraction	qPCR	5	MTB Not Detected			Undetermined	
376	2.21.21	Qiagen Extraction	qPCR	0	MTB Not Detected			Undetermined	
377	2.21.21	Qiagen Extraction	qPCR	100	MTB Detected			29.82893753	
378	2.21.21	Qiagen Extraction	qPCR	5	MTB Not Detected			Undetermined	
379	2.21.21	Qiagen Extraction	qPCR	5	MTB Not Detected (Ct>38)			40.15118408	
380	2.21.21	Qiagen Extraction	qPCR	5	MTB Detected			34.48693848	
381	2.21.21	Qiagen Extraction	qPCR	50	MTB Detected			31.29413033	
382	2.21.21	Qiagen Extraction	qPCR	10	MTB Detected			33.09703064	
383	2.21.21	Qiagen Extraction	qPCR	50	MTB Detected			32.18887329	
384	2.21.21	Qiagen Extraction	qPCR	50	MTB Detected			31.29738235	
385	2.21.21	Qiagen Extraction	qPCR	5	MTB Not Detected			Undetermined	
386	2.21.21	Qiagen Extraction	qPCR	5	MTB Not Detected			Undetermined	
387	2.21.21	Qiagen Extraction	qPCR	10	MTB Detected			37.18040466	
388	2.21.21	Qiagen Extraction	qPCR	50	MTB Detected			36.37857819	
389	2.21.21	Qiagen Extraction	qPCR	0	MTB Not Detected			Undetermined	
390	2.21.21	Qiagen Extraction	qPCR	50	MTB Detected			31.05113792	
391	2.21.21	Qiagen Extraction	qPCR	0	MTB Not Detected			Undetermined	
392	2.21.21	Qiagen Extraction	qPCR	10	MTB Not Detected (Ct>38)			40.31593704	
393	2.21.21	Qiagen Extraction	qPCR	50	MTB Detected			33.07100677	
394	2.21.21	Qiagen Extraction	qPCR	10	MTB Not Detected			Undetermined	
395	2.21.21	Qiagen Extraction	qPCR	0	MTB Detected			34.98595428	
396	2.21.21	Qiagen Extraction	qPCR	5	MTB Not Detected			Undetermined	
397	2.21.21	Qiagen Extraction	qPCR	100	MTB Detected			31.1156826	
398	2.21.21	Qiagen Extraction	qPCR	0	MTB Not Detected			Undetermined	
399	2.21.21	Qiagen Extraction	qPCR	0	MTB Not Detected			Undetermined	
400	2.21.21	Qiagen Extraction	qPCR	50	MTB Detected			33.15472794	

Sample #	Date Run	Conditions	Method 1-3, qPCR, Air, Saliva, Other	CFU/Swab	Diagnosis	Lowest rpoB (C_T)	IS1081/6110 (C_T)	IS6110 (C_T)	Error Type
401	2.21.21	Qiagen Extraction	qPCR	50	MTB Detected			33.76051331	
402	2.21.21	Qiagen Extraction	qPCR	100	MTB Detected			31.9558239	
403	2.21.21	Qiagen Extraction	qPCR	10	MTB Not Detected			Undetermined	
404	2.21.21	Qiagen Extraction	qPCR	100	MTB Detected			30.49729919	
405	2.21.21	Qiagen Extraction	qPCR	5	MTB Not Detected			Undetermined	
406	2.21.21	Qiagen Extraction	qPCR	10	MTB Not Detected (Ct>38)			40.94535828	
407	2.21.21	Qiagen Extraction	qPCR	5	MTB Not Detected			Undetermined	
408	2.21.21	Qiagen Extraction	qPCR	0	MTB Not Detected			Undetermined	
409	2.21.21	Qiagen Extraction	qPCR	50	MTB Detected			33.37908554	
410	2.21.21	Qiagen Extraction	qPCR	10	MTB Detected			37.43810272	
411	2.21.21	Qiagen Extraction	qPCR	50	MTB Detected			31.83530998	
412	2.21.21	Qiagen Extraction	qPCR	100	MTB Detected			30.40890694	
413	2.21.21	Qiagen Extraction	qPCR	10	MTB Not Detected (Ct>38)			38.38638687	
414	2.21.21	Qiagen Extraction	qPCR	0	MTB Not Detected			Undetermined	
415	2.21.21	Qiagen Extraction	qPCR	100	MTB Detected			30.02602577	
416	2.21.21	Qiagen Extraction	qPCR	100	MTB Detected			29.20711708	