Sample adequacy controls for infectious disease diagnosis by oral swabbing

Meagan Deviaene¹, Kris M. Weigel¹, Rachel C. Wood¹, Angelique K. K. Luabeya², Lisa Jones-Engel¹, Mark Hatherill², Gerard A. Cangelosi¹*

¹ Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington, United States of America, ² Department of Pathology, South African Tuberculosis Vaccine Initiative (SATVI), Institute of Infectious Disease & Molecular Medicine and Division of Immunology, University of Cape Town, Cape Town, South Africa

* gcang@uw.edu

Abstract

Oral swabs are emerging as a non-invasive sample type for diagnosing infectious diseases including Ebola, tuberculosis (TB), and COVID-19. To assure proper sample collection, sample adequacy controls (SACs) are needed that detect substances indicative of samples collected within the oral cavity. This study evaluated two candidate SACs for this purpose. One detected representative oral microbiota (Streptococcus species DNA) and the other, human cells (human mitochondrial DNA, mtDNA). Quantitative PCR (qPCR) assays for the two target cell types were applied to buccal swabs (representing samples collected within the oral cavity) and hand swabs (representing improperly collected samples) obtained from 51 healthy U.S. volunteers. Quantification cycle (Cq) cutoffs that maximized Youden’s index were established for each assay. The streptococcal target at a Cq cutoff of \( \leq 34.9 \) had 99.0% sensitivity and specificity for oral swab samples, whereas human mtDNA perfectly distinguished between hand and mouth swabs with a Cq cutoff of 31.3. The human mtDNA test was then applied to buccal, tongue, and gum swabs that had previously been collected from TB patients and controls in South Africa, along with “air swabs” collected as negative controls (total N = 292 swabs from 71 subjects). Of these swabs, 287/292 (98%) exhibited the expected Cq values. In a paired analysis the three oral sites yielded indistinguishable amounts of human mtDNA, however PurFlock™ swabs collected slightly more human mtDNA than did OmniSwabs™ (\( p = 0.012 \)). The results indicate that quantification of human mtDNA cannot distinguish swabs collected from different sites within the mouth. However, it can reliably distinguish oral swabs from swabs that were not used orally, which makes it a useful SAC for oral swab-based diagnosis.

Introduction

Oral swabbing is a non-invasive, inexpensive, and easy-to-use sampling strategy for infection diagnosis. An oral swab sample is collected by brushing a swab on a surface within the mouth, such as the tongue dorsum or buccal mucosa (inner cheek). Oral swabs have been employed in experimental or clinical contexts to detect Ebola virus, human papillomavirus, Mycobacterium
tuberculosis, Leishmania, the novel coronavirus SARS-CoV-2, and other infectious diseases in both humans and animals [1–11]. Compared to more traditional specimens such as sputum, urine, blood, and nasopharyngeal swabs, oral swabbing is faster, less invasive, and requires no accommodations for privacy or aerosol control. In the case of TB, recent studies showed that qPCR analysis of oral swabs can detect Mycobacterium tuberculosis DNA in up to 93% of sputum qPCR-positive adult pulmonary TB patients [1,2,7]. One of these studies showed that tongue swabs yield significantly better M. tuberculosis signal than cheek or gum swabs [1]. In another recent study, self-collected tongue swabs detected 47/50 patients with confirmed COVID-19, for a sensitivity of 90% relative to the more invasive nasopharyngeal swabbing method [11]. Such results illustrate the expanding potential for simple swabbing methods in the molecular diagnosis of infectious diseases, and the need for additional research in this area.

One hindrance to the evaluation and implementation of oral swab methods is the potential for false negative results due to inadequate sampling (insufficient contact with oral mucosa). This problem is especially acute in the context of self-sampling as envisioned for scaled-up COVID-19 screening [11,12]. In contrast to samples such as sputum and blood, which can be inspected visually to assure collection, samples collected within the mouth are most often visually indistinguishable from those which have never been inside a mouth. This challenge can be met by using a sample adequacy control (SAC), defined in this study and elsewhere [13] as a molecular target which differs from the diagnostic target and should consistently be present in definable amounts in properly collected specimens.

Though standards for evaluating diagnostic tests frequently include quality assurance (QA) strategies [14–16] components and levels of QA are differentially adopted. Often, reference materials are added to samples as positive controls, for example purified DNA [17]. These are good practices to validate laboratory processes but they do not address the upstream issue of sampling adequacy. Even among approved molecular diagnostic assays, use of sample adequacy controls is not uniform [18]. In some cases, SACs are successfully utilized without preliminary validation. For example, in recent reports on the successful use of oral swabbing and virus nucleic acid testing for Ebola virus, endogenous controls targeting human nucleic acids were used as SACs. However, no determination of the amount of such nucleic acids that constitutes a swab collected within the oral cavity were presented [3,4].

The present study evaluated two qPCR assays for their potential use in verifying whether a swab has sampled material within the mouth. One candidate SAC exploited the expanding understanding of the human oral microbiome in order to design a qPCR test specific for some of these microbes. The oral cavity has a diverse microbiota, with over 700 species identified [19], yet among human microbiomes it has one of the lowest levels of beta diversity [20]. Streptococcus is a core genus of human oral microbiomes [21] and one of our candidate SAC tests targeted species of this genus.

The second candidate SAC test targeted a ubiquitous human mitochondrial gene, under the assumption that swabs with sufficient contact with the oral epithelium will consistently contain more human cells than swabs which had been handled but not had contact with the oral cavity. Human mitochondrial DNA (mtDNA) is comprised of the 37 genes encoded within the cellular organelle. MtDNA is suitable for use as SACs in infectious disease diagnostic tests as they are always present in detectable quantities, and polymorphisms within these genes are rare and mitigated by the heteroplasmy of mtDNA [22,23]. Most often, housekeeping genes have been employed to ensure that inhibition is not occurring in the qPCR amplification, rather than for the purpose of ascertaining sampling adequacy [17,24,25]. MtDNA was selected over other housekeeping genes because it was used previously in studies on non-human primates [9].
To validate the candidate SACs, a study was undertaken that was paired in two ways. First, hand and mouth swabs were tested from each individual. Second, two qPCR assays (for *Streptococcus* species and for human mtDNA) were performed on each sample. Hand swabs were used as simulated mishandled swabs that incidentally contacted human skin outside of the mouth. Such swabs were expected to contain both human and streptococcal DNA, but in smaller amounts than mouth swabs. We tested the hypothesis that mouth swab Cq values are sufficiently different from hand swab Cq values to enable the establishment of a threshold value separating the two groups. After optimization, a method utilizing human mtDNA was then applied to oral swabs collected by diverse methods from a cohort in Worcester, South Africa, as part of a recently completed analysis of oral swab testing for testing for TB [1].

**Materials and methods**

**Sample populations**

Subjects in Seattle, WA, USA were recruited via flyers and emails that were posted and sent throughout the University of Washington campus. Samples were collected from subjects in their workplaces. Samples were stored at -80°C within 8 hours of collection, and subsequently tested by qPCR as described below.

Samples archived as part of a previous study on the use of oral swabs in the diagnosis of adult pulmonary TB patients in South Africa [1] were also used for the current study. South African participants had been recruited in clinics in Worcester, Western Cape (population 350,000). As described previously, these samples were collected from subjects in clinics or homes, stored at -80°C within 8 hours of collection, and shipped frozen to Seattle where they were assayed by qPCR for *M. tuberculosis* DNA [1]. For the current study these archived samples were screened for human mtDNA.

Protocols for collecting samples from U.S. subjects were approved by the University of Washington’s Human Subjects Division. Archived South African samples collected for the previous study focusing on TB diagnosis [1] were used in the current study under “future use” provisions in consent forms, as approved by the University of Washington and the University of Cape Town.

**Swab collection**

In Seattle, samples were collected by brushing Whatman OmniSwabs™ along the inside of each subject’s cheek (oral swabs) or the surfaces of the palm and fingers (hand swabs) 7–8 times, or about 5 seconds.

In South Africa, OmniSwabs™ and Puritan PurFlock™ swabs were previously collected by brushing the inner surface of the tongue dorsum, gums, or cheeks for 10 seconds [1]. OmniSwabs™ are a more expensive paper swab with a serrated head as compared to PurFlock™ swabs whose heads are composed of Polyester Flock and are rounded. These two swab types were being compared as a part of a previous study [1]. “Air controls” were collected at both study sites by exposing a swab to air for 10 seconds (PurFlock™ in South Africa, OmniSwab™ in Seattle). Immediately following sampling, all swab heads were deposited into, individual 500 mL tubes containing a lysis/preservation buffer (pH 8.0) consisting of 50 mM EDTA and sucrose, 100 mM sodium chloride, 65 mM tris, and 0.3% SDS. Samples were stored frozen at -80°C until use.

For the present study a total of 292 South African swabs were tested. They were selected based on the availability of partially processed samples, at a time point in the previous study [1] when TB status was blinded to us. The 292 swabs came from 71 South African subjects, each of whom provided up to 5 swabs (4 oral and 1 air). Some subjects had fewer than 5 swabs tested for this study. Only subjects for whom all 4 oral swabs were tested (N = 32) were...
included in comparisons between swabbing sites and swab brands. After unblinding, this cohort was found to include 7 TB-positive subjects and 25 TB-negative subjects.

**DNA extraction**

Samples collected from U.S. volunteers were extracted using the QIAGEN 96 DNeasy Blood and Tissue Kit protocol. To begin extraction samples were boiled in lysis/preservation buffer at 100˚C for 10 min, followed by addition of proteinase K and QIAGEN lysis buffer AL and a 10-min incubation at 56˚C. Ethanol was then added to the samples and they were transferred to a plate column well for binding, two washes and elution. Nucleic acid was extracted from swab samples collected in South Africa using a modified spin column protocol of the QIAGEN QIAamp DNA mini kit (catalog #51306), described previously [1].

**Quantitative PCR**

Each U.S. sample was tested twice, once for streptococcal DNA and once for human mtDNA (Table 1). The *Streptococcus* species qPCR was comprised of primers from Picard et al [26] with the addition of a hydrolysis probe falling within the amplicon of the two primers, and designed for this study to be specific for four species of *Streptococcus*, namely *S. gordonii*, *S. mitis*, *S. pneumoniae*, and *S. sanguinis*. These species were selected because of their established presence in the oral microbiome, combined with a limited known presence on the skin or in the environment, as reported by the Human Microbiome Project (hmpdacc.org/HMRGD). Design of the primer was accomplished using NCBI Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and Primer 3 (http://bioinfo.ut.ee/primer3-0.4.0/). The buffer and primer concentrations were identical to the published protocol [26], with the modifications of probe concentration at 0.5 μM in the final reaction mixture and 2 μL of template instead of 1 μL. The human mtDNA qPCR was adapted from Caldwell et al. [27] qPCR primer and probe sequences as well as thermocycling conditions are summarized in Table 1. For the samples from South Africa, 5 μL of archived extracted DNA from the previous study [1] was tested only by the assay targeting human mtDNA.

**Statistical analyses**

Calculations were carried out using R, version 3.1.3. The package OptimalCutpoints was used to calculate Cq cutoffs with Youden’s index. T-tests for both Seattle and South African studies were performed on paired samples because they evaluated within-subject differences.

**Results**

**Comparison of two SAC targets**

We first compared streptococcal DNA and human mtDNA as markers for oral swab SAC. While both markers were assumed to be ubiquitous on human skin and in the environment,
we hypothesized that swabs with sufficient contact with the oral cavity would consistently have more of these materials than swabs that had not been inside human mouths. To test this, 51 healthy U.S. subjects were enrolled and 5 OmniSwabs™ were collected per participant: One from each hand, one from each inner cheek, and one “air control” swab, which constitutes a swab exposed to air for 10 seconds to serve as a negative control. Cq values (inversely proportional to target quantity) from streptococcal and human mtDNA qPCR tests conducted on all 255 samples (n = 510) were compared.

Results from qPCR analyses of mouth and hand swabs are summarized in Fig 1. A Cq cutoff value that maximized Youden’s index ([(sensitivity + specificity) - 1]) was established for each assay. The qPCR assay for streptococcal DNA had an optimal Cq cutoff at 34.9. Cq values below this cutoff corresponded with oral swabbing with a sensitivity of 99.0% and a specificity of 99.0%. Thus, the streptococcal assay was imperfect as a SAC. In contrast, human mtDNA as a classifier was able to perfectly distinguish between hand and mouth samples with a threshold Cq value of 31.3 (Fig 1). Air swabs yielded no detectable qPCR signal for either human mtDNA or streptococcal DNA in 94% of cases (48/51). When qPCR signals were observed in air swabs, their Cq values were always above the optimal cutoff value.

**Comparison of oral sites and swab brands**

Next, the human mtDNA sample adequacy control was applied to samples previously collected in a blinded study from South African TB patients and controls [1]. In Phase 1 of the previous study, South Africans with possible TB sampled by oral swabbing. Swabs were collected from each subject on three different days. For the present study, a total of 292 swabs from 71 South African subjects were tested for human mtDNA. The oral site and swab brand comparisons focused on oral swabs collected from 32 participants during their second swabbing session (Day 2). The swabs were collected from three different oral sites using two different swab brands, specifically: Tongue dorsum (OmniSwabs™), gum (OmniSwabs™), right buccal surface (OmniSwabs™), and left buccal surface (PurFlock™) [1].

![Fig 1. Oral swabs, hand swabs, and air swabs collected in the US. Data points are Cq values (inversely proportional to target DNA quantity) from qPCR analyses of cheek, hand, and air swab samples from 51 healthy U.S. subjects. Each sample was tested for streptococcal (Strep) DNA and human mtDNA. Samples with no detectable qPCR signal were assigned Cq values of 40.](https://doi.org/10.1371/journal.pone.0241542.g001)
Cq values from buccal OmniSwab™ samples collected from U.S. and South African donors (Figs 1 and 2, respectively) were compared in a T-test for differences in human mtDNA. Buccal OmniSwab™ Cq values from the South African cohort were significantly lower (H_A = two-sided) than those from the initial evaluation done on the U.S. cohort (p = 0.0016).

Comparing mouth regions, in a paired analysis Cq values for gum or tongue OmniSwabs™ were not significantly different from buccal OmniSwabs™ (Fig 2). Comparing swab types, PurFlock™ buccal swabs yielded lower Cq values (indicating more human mtDNA) than OmniSwab™ buccal swabs (p = 0.019) (Fig 2). Thus, it appears that PurFlock™ swabs collect slightly more human biological material than OmniSwabs™ in the same mouth region.

The South African sample did not include hand swabs, however it did include PurFlock™ “air swabs” that were collected in South African homes and clinics at the same time that oral swabs were collected. The human mtDNA test was applied to a sample of 64 of these swabs, 20 of which were collected at the same time and location as the oral swabs in Fig 2. Human mtDNA was detected in many of these swabs. However, Cq values always fell above the threshold value of 31.3, indicative of orally collected swabs (Fig 2).

Discussion

Oral swabs are emerging as non-invasive samples for diagnosing infectious diseases. In the most sensitive protocol identified to date for oral swab diagnosis of TB [1], the dorsum of the tongue is gently scraped and the swab head with collected material, consisting of bacterial biofilm and host cells, is deposited into a sample buffer; the sample is a semi-clear, non-viscous suspension suitable for testing by qPCR targeting M. tuberculosis DNA. Tongue swabbing is
fast, painless, and does not require accommodations for privacy or aerosol control. Sputum-scarce patients such as children and HIV-positive adults are easily swabbed. In recent studies the method approached the sensitivity and specificity of sputum testing in adults [1]. In a separate study, the method approached the sensitivity and specificity of gold-standard nasopharyngeal swabbing for the detection of COVID-19 [3].

Despite these advantages, additional work is needed to bring the method into parity with traditional methods. For example, in one study buccal swabs collected from TB patients exhibited only 45% sensitivity relative to sputum testing [7]. In that study swabs were tested using the Cepheid GeneXpert Ultra using a protocol designed for sputum (not swab) sample processing and testing. Moreover, it used buccal swabs [2], not the more optimal tongue swabs [1]. Such results support the feasibility of oral swabbing for TB, but highlight the need for more research to yield fully optimized methods. As such work proceeds, it is essential to control for possible false negative results that arise from inadequate sampling. In contrast to visually distinctive specimens such as sputum and blood, a swab collected in the oral cavity can resemble a swab that has never been inside a mouth. Therefore, the current study identified and evaluated SACs for use in evaluating and implementing oral swab-based diagnostic methods.

While tests targeting human mtDNA and streptococcal DNA both appeared able to distinguish mouth swabs from hand swabs, the human mtDNA test was more sensitive and specific at a calculated Cq cutoff. This was largely due to the more variable and higher overall Cq values observed when mouth swabs were tested by the streptococcal assay. This observation is consistent with the beta diversity that has been reported for human oral microbiomes [22–24].

Human mtDNA signals in buccal swabs from the South African cohort were significantly lower than those from the U.S. cohort. This difference may reflect disparities in sampling or DNA extraction methods. Swabbing duration for South African participants was 10 seconds, longer than for the participants in the Seattle phase of this study (5 seconds). Different QIA-GEN extraction kits were utilized for samples deriving from the different sites.

It is noteworthy that a significant number of air swabs had positive signals for human DNA, albeit well outside the threshold Cq value. The number was larger in the South African sample than in the U.S. sample, possibly because samples were collected in more diverse environments in South Africa, including clinics and patient homes. Human DNA is ubiquitous in built environments [28]. This underscores the need to use quantitative methods combined with a well-validated threshold value when human DNA is used as a SAC.

All U.S. oral swab samples fell below the human mtDNA Cq threshold for orally collected swabs. In the South African sample, 5/292 oral swabs (1.71%) fell above the Cq threshold established in this study. This included 3 buccal swabs, one gum swab, and one tongue swab. These samples were excluded from the previous study [1].

The human mtDNA qPCR appears to be robust and useful as a SAC for determining whether a swab has been collected in several mouth regions using 2 swab brands. Nonetheless, some quantitative variations were observed. The South African samples had lower Cq values (more human mtDNA) than the U.S. samples. This might reflect differences in sampling methods, prevalence of TB, and/or comorbidities, diet, socioeconomic factors, and/or microbiomes.

PurFlock™ buccal swabs yielded lower Cq values (indicating more human mtDNA) than OmniSwab™ (p = 0.035). This finding is consistent with improved performance of that swab type in detecting TB [1]. This may reflect a higher capacity for human biological material.

This study had several limitations. The sample size was limited and restricted to two geographical locations in the U.S. and South Africa. The South African sample was a convenience sample consisting of people visiting a clinic for TB-like symptoms. Although it included air
swabs, it did not include hand swabs. The current analysis was non-blinded. Neither human mtDNA nor Streptococcal DNA were found to be useful for distinguishing swabs collected from different sites within the mouth.

Despite these limitations, the results indicate that PCR for human mtDNA can reliably distinguish oral swabs from swabs that were not used orally. This feature makes it a useful SAC for oral swab-based diagnosis. The human mtDNA SAC may prove useful in the evaluation and implementation of oral swabs as non-invasive tools for infectious disease diagnosis.

**Acknowledgments**

The authors wish to thank Felicia Nguyen and Alaina Olson, who aided in sample analysis, and volunteers who provided samples for this project. We are also grateful to the staff at SATVI in South Africa for their role in recruiting and sampling participants at that site.

**Author Contributions**

**Conceptualization:** Meagan Deviaene, Kris M. Weigel, Rachel C. Wood, Angelique K. K. Luabeya, Lisa Jones-Engel, Gerard A. Cangelosi.

**Data curation:** Rachel C. Wood, Gerard A. Cangelosi.

**Formal analysis:** Meagan Deviaene, Kris M. Weigel, Rachel C. Wood, Lisa Jones-Engel, Gerard A. Cangelosi.

**Funding acquisition:** Gerard A. Cangelosi.

**Investigation:** Meagan Deviaene, Kris M. Weigel, Rachel C. Wood, Gerard A. Cangelosi.

**Methodology:** Meagan Deviaene, Kris M. Weigel, Rachel C. Wood, Angelique K. K. Luabeya, Lisa Jones-Engel, Gerard A. Cangelosi.

**Project administration:** Mark Hatherill, Gerard A. Cangelosi.

**Supervision:** Kris M. Weigel, Mark Hatherill, Gerard A. Cangelosi.

**Validation:** Meagan Deviaene, Kris M. Weigel, Mark Hatherill, Gerard A. Cangelosi.

**Visualization:** Kris M. Weigel, Rachel C. Wood, Mark Hatherill, Gerard A. Cangelosi.

**Writing – original draft:** Meagan Deviaene, Rachel C. Wood, Gerard A. Cangelosi.

**Writing – review & editing:** Meagan Deviaene, Kris M. Weigel, Rachel C. Wood, Angelique K. K. Luabeya, Lisa Jones-Engel, Mark Hatherill, Gerard A. Cangelosi.

**References**

1. Luabeya AK, Wood RC, Shenje J, Filander E, Ontong C, Mabwe S, et al. Noninvasive Detection of Tuberculosis by Oral Swab Analysis. J Clin Microbiol [Internet]. 2019 Mar 1 [cited 2020 Mar 20]; 57(3). Available from: http://www.ncbi.nlm.nih.gov/pubmed/30541931. https://doi.org/10.1128/JCM.01847-18 PMID: 30541931

2. Wood RC, Luabeya AK, Weigel KM, Wilbur AK, Jones-engel L, Hatherill M, et al. Detection of Mycobacterium tuberculosis DNA on the oral mucosa of tuberculosis patients. 2015;1–5.

3. Semper AE, Broadhurst MJ, Richards J, Foster GM, Simpson AJH, Logue CH, et al. Performance of the GeneXpert Ebola Assay for Diagnosis of Ebola Virus Disease in Sierra Leone: A Field Evaluation Study. PLoS Med [Internet]. 2016 Mar 1 [cited 2020 Mar 20]; 13(3):e1001980. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27023868. https://doi.org/10.1371/journal.pmed.1001980 PMID: 27023868

4. Erickson BR, Sealy TK, Flietstra T, Morgan L, Kargbo B, Matt-Lebby VE, et al. Ebola Virus Disease Diagnostics, Sierra Leone: Analysis of Real-time Reverse Transcription-Polymerase Chain Reaction Values for Clinical Blood and Oral Swab Specimens. J Infect Dis [Internet]. 2016 Oct 15 [cited 2016 Oct...
Sample adequacy controls for oral swab diagnostics

14; 214(suppl 3):S258–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27587631. https://doi.org/10.1128/JCM.01463-09 PMID: 19864480

5. Aschar M, de Oliveira ETB, Laurenti MD, Marcondes M, Tolezano JE, Hiramoto RM, et al. Value of the oral swab for the molecular diagnosis of dogs in different stages of infection with Leishmania infantum. Vet Parasitol [Internet]. 2016 Jul 30 [cited 2016 Oct 14]; 225:108–13. Available from: http://www.ncbi.nlm.nih.gov/pubmed/27369584. https://doi.org/10.1016/j.vetpar.2016.06.005 PMID: 27369584

6. Hang D, Liu F, Liu M, He Z, Sun M, Liu Y, et al. Oral human papillomavirus infection and its risk factors among 5,410 healthy adults in China, 2009–2011. Cancer Epidemiol Biomarkers Prev. 2014; 23 (10):2101–10. https://doi.org/10.1158/1055-2996.EPI-14-0084 PMID: 25033824

8. Nicol MP, Wood RC, Workman L, Prins M, Whitman C, Ghebrekristos Y, et al. Microbiological diagnosis of pulmonary tuberculosis in children by oral swab polymerase chain reaction. Sci Rep. 2019 Dec 1; 9 (1). https://doi.org/10.1038/s41598-019-47302-5 PMID: 31342525

9. Engel GA, Wilbur AK, Westmark A, Horn D, Johnson J, Jones-Engel L. Naturally acquired Mycobacterium tuberculosis complex in laboratory pig-tailed macaques. Emerg Microbes Infect [Internet]. 2012; 1 (10):e30. Available from: http://www.nature.com/dofinder/10.1038/emi.2012.31. PMID: 26038402

10. Wilbur AK, Engel GA, Rompis A, Putra IGAA, Lee BPYH, Aggiramarangse N, et al. From the Mouths of Monkeys: Detection of Mycobacterium tuberculosis Complex DNA From Buccal Swabs of Synanthropic Macaques. Am J Primatol. 2012; 74(7):676–86. https://doi.org/10.1002/ajp.22022 PMID: 22645480

11. Tu Y-P, Jennings R, Hart B, Cangelosi GA, Wood RC, Wehber K, et al. Swabs Collected by Patients or Health Care Workers for SARS-CoV-2 Testing. N Engl J Med [Internet]. 2020 Jun 3 [cited 2020 Jun 23]; Available from: https://pubmed.ncbi.nlm.nih.gov:8080/32492294/

12. Kim AE, Brandstetter E, Graham C, Heinonen J, Osterb ind A, McCulloch DJ, et al. Seattle Flu Study—Swab and Send: Study Protocol for At-Home Surveillance Methods to Estimate the Burden of Respiratory Pathogens on a City-Wide Scale. medRxiv. 2020 Mar 7;2020.03.04.20031211.

13. Brukner I, Eintracht S, Papadakis AI, Faucher D, Lamontagne B, Spatz A, et al. Maximizing confidence in a negative result: Quantitative sample adequacy control. J Infect Public Health. 2020 Jul 1; 13 (7):991–3. https://doi.org/10.1016/j.jiph.2020.01.307 PMID: 32037201

14. Banoo S, Bell D, Bossuyt P, Herring A, Mabey D, Poole F, et al. Evaluation of diagnostic tests for infectious diseases: General principles. Nat Rev Microbiol. 2006; 4(12 SUPPL.):21–33.

15. Kosack CS, Page A-L, Klatser PR. A guide to aid the selection of diagnostic tests. Bull World Health Organ [Internet]. 2017 Sep 1 [cited 2020 Mar 20]; 95(9):639–45. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28867844. https://doi.org/10.1093/bmj/BLT.16.187468 PMID: 28867844

16. Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, et al. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol [Internet]. 2010 Jan [cited 2020 Mar 20]; 48(1):229–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19864480. https://doi.org/10.1128/JCM.01453-09 PMID: 19864480

17. Espy MJ, Uhl JR, Sloan LM, Buckwalter SP, Jones MF, Vetter EA, et al. Real-Time PCR in Clinical Microbiology: Applications for Routine Laboratory Testing. Clin Microbiol Rev. 2006; 19(1):165–256. https://doi.org/10.1128/CMR.19.1.165-256.2006 PMID: 16418529

18. Emmadi R, Boonyaratankomkit JB, Selvarangan R, Shyamala V, Zimmer BL, Williams L, et al. Molecular methods and platforms for infectious diseases testing: A review of FDA-approved and cleared assays. J Mol Diagnostics [Internet]. 2011; 13(6):583–604. Available from: https://doi.org/10.1016/j.jmoldx.2011.05.011 PMID: 21871973

19. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the Normal Bacterial Flora of the Oral Cavity Defining the Normal Bacterial Flora of the Oral Cavity. J Clin Microbiol. 2005; 43(11):5721–32. https://doi.org/10.1128/JCM.43.11.5721-5732.2005 PMID: 16272510

20. Huttonhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, et al. Structure, function and diversity of the healthy human microbiome. Nature [Internet]. 2012; 486(7402):207–14. Available from: http://www.nature.com/dofinder/10.1038/nature11234. PMID: 22699609

21. Li K, Bihan M, Methé BA. Analyses of the Stability and Core Taxonomic Memberships of the Human Microbiome. PLoS One. 2013; 8(5). https://doi.org/10.1371/journal.pone.0063139 PMID: 23671663

22. Tuppen HAL, Blakely EL, Turnbull DM, Taylor RW. Mitochondrial DNA mutations and human disease. Biochim Biophys Acta—Bioenerg [Internet]. 2010 [cited 2017 May 15]; 1797(2):113–28. Available from: http://www.sciencedirect.com/science/article/pii/S0009282910002618. https://doi.org/10.1016/j.bbabio.2009.09.005 PMID: 19781752
23. Wallace DC, Chalkia D. Mitochondrial DNA genetics and the heteroplasmic conundrum in evolution and disease. Cold Spring Harb Perspect Biol [Internet]. 2013 [cited 2017 May 15]; 5(11). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3809581/pdf/cshperspect-MIT-a021220.pdf.

24. Sepúlveda D, Bohle H, Labra Á, Grotthusen H, Marshall SH, Schreck C, et al. Design and evaluation of a unique RT-qPCR assay for diagnostic quality control assessment that is applicable to pathogen detection in three species of salmonid fish. BMC Vet Res [Internet]. 2013 [cited 2016 Oct 18]; 9(1):183. Available from: http://bmcvetres.biomedcentral.com/articles/10.1186/1746-6148-9-183. https://doi.org/10.1186/1746-6148-9-183 PMID: 24040749

25. Pugnale P, Latorre P, Rossi C, Crovatto K, Pazienza V, Gottardi A De, et al. Real-time multiplex PCR assay to quantify hepatitis C virus RNA in peripheral blood mononuclear cells. J Virol Methods [Internet]. 2006 May [cited 2016 Oct 18]; 133(2):195–204. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16384611. https://doi.org/10.1016/j.jviromet.2005.11.007 PMID: 16384611

26. Picard FJ, Ke D, Boudreau DK, Boissinot M, Huletsky A, Richard D, et al. Use of tuf sequences for genus-specific PCR detection and phylogenetic analysis of 28 streptococcal species. J Clin Microbiol. 2004; 42(8):3686–95. https://doi.org/10.1128/JCM.42.8.3686-3695.2004 PMID: 15297518

27. Caldwell JM, Raley ME, Levine JF, Levine J a YF. Mitochondrial Multiplex Real-Time PCR as a Source Tracking Method in Fecal-Contaminated Effluents. Mitochondrial Multiplex Real-Time PCR as a Source Tracking Method in Fecal-Contaminated Effluents. Environ Sci Technol. 2007; 41(9):3277–83. https://doi.org/10.1021/es062912s PMID: 17539537

28. Afshinneko E, Meydan C, Chowdhury S, Jaroudi D, Boyer C, Bernstein N, et al. Geospatial Resolution of Human and Bacterial Diversity with City-Scale Metagenomics. Cell Syst. 2015 Jul 29; 1(1):72–87. https://doi.org/10.1016/j.cels.2015.01.001 PMID: 26594662