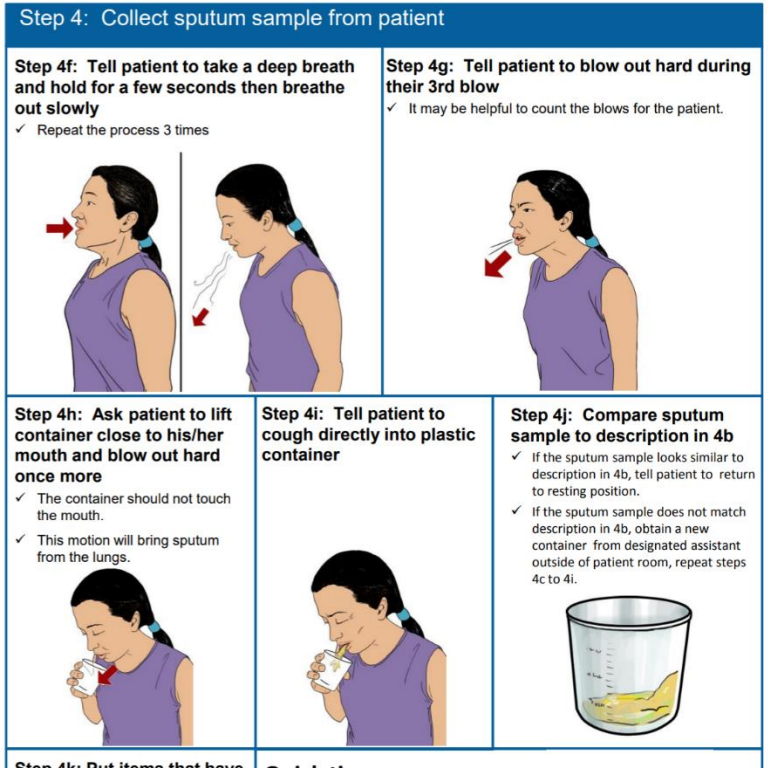
Alternatives to sputum testing for detection of TB in clinical and community settings

Jerry Cangelosi, Ph.D.
Department of Environmental and Occupational Health Sciences

UNIVERSITY of WASHINGTON

TRTC TUBERCULOSIS RESEARCH & TRAINING CENTER
UNIVERSITY of WASHINGTON

Microbiological diagnosis of pulmonary tuberculosis: Collection and testing of sputum



WHO

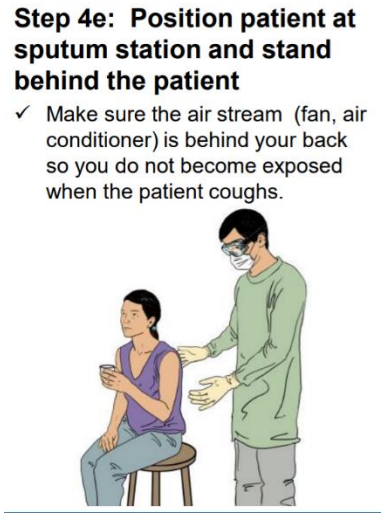
Sputum smear microscopy

Sputum culture

Nucleic acid amplification
e.g. Cepheid GeneXpert
MTB/RIF Ultra

Why look beyond sputum?

- Occupational safety for healthcare workers
- Some patients can't always provide sputum (e.g. HIV coinfected, children)
- Sputum is viscous, non-uniform, difficult to process and analyze
- Logistically difficult to collect sputum in community settings



"…the need for a biomarker-based, low-cost, non-sputum-based test remains a key priority for TB diagnostics beyond the microscopy centre."
-2014 UNITAID. Tuberculosis diagnostics technology and market landscape - 3rd edition. World Health Organization.

"…the application of twenty first century diagnostic technologies that can detect Mtb in a variety of clinical specimens from multiple body sites in addition to sputum, as well as advanced approaches for monitoring and predicting treatment outcomes are a priority."
-Fauci AS and Eisinger RW (2018). Reimagining the Research Approach to Tuberculosis. Am. J. Trop. Med. Hyg., 98:650–652

Non-invasive swab sampling for SARS-CoV-2: A parable for finding the "missing millions" of TB cases

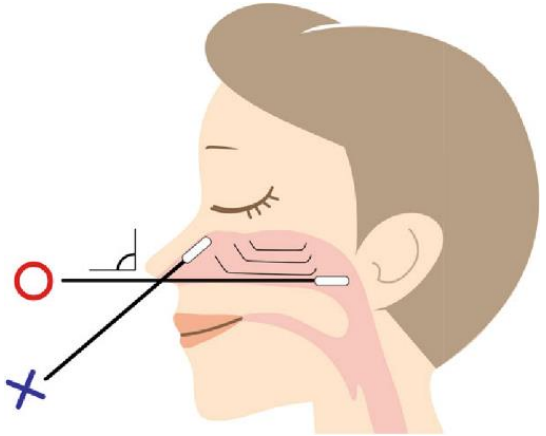
The NEW ENGLAND JOURNAL of MEDICINE

CORRESPONDENCE

Swabs Collected by Patients or Health Care Workers for SARS-CoV-2 Testing

Tu YP, Jennings R, Hart B, Cangelosi GA, Wood RC, Wehber K, Verma P, Vojta D, Berke EM. N Engl J Med. 2020 Jul 30;383(5):494-496. PMC7289274.

Nasal vs. nasopharyngeal swabbing (Louisiana Dept. of Health, 2020)



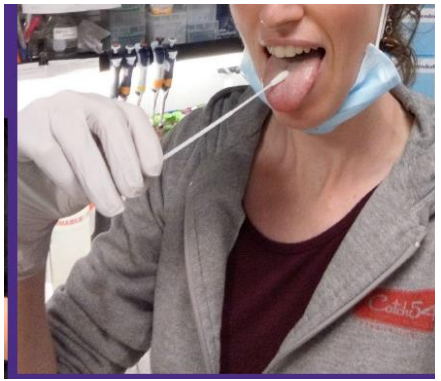
2022

Alternative samples for TB case finding: Some examples

- Host biomarkers
- Urine (LAM, DNA)
- Stool
- Oral swabs
- Exhaled breath (samplers, face masks)
- Acoustic monitoring of coughs

TB diagnosis by oral swab analysis

- Scrape tongue dorsum ~5 seconds, eject swab head into transport buffer (or dry)
- Sample = bacterial biofilm, host cells
  - Not saliva
- Tongue swabbing better than cheek or gum swabbing (Luabeya et al, 2019)
- Detect *M. tuberculosis* DNA by qPCR or other methods
- Anyone can be sampled in seconds
  - Easy self-sampling
  - TB symptoms (sputum production) not required



Oral Swab Analysis (OSA): Evaluations in adult pulmonary TB

Oral site	Swab	Sens relative to sputum Xpert® MTB/RIF	Sens relative to all TB cases	Spec relative to ill non-TB & healthy controls	Site
Buccal (cheek)	Whatman OmniSwab 3 swabs/subject	18/20 (90%)	ND	20/20 (100%)	South Africa, USA (Wood et al 2015)
Tongue dorsum	Puritan Purflock 2 swabs/subject	128/138 (93%)	49/59 (83%)	65/71(92%)	South Africa (Luabeya et al 2019)
Tongue dorsum	Copan FLOQswab 1 swab/subject	61/68 (90%)	ND	41/53 (77%)	Uganda (Wood et al 2021)

Lisa Jones-Engel

Oral swab testing of pediatric TB

Nicol M et al 2019

- **Manual IS6110 qPCR**
- **Reference standard: 2X induced sputum culture**
- **OSA was insensitive in sputum-positive children (“confirmed TB”)**
- **But it detected many children with TB who were sputum-negative (“unconfirmed TB”)**

	Confirmed TB	Unconfirmed TB	Unlikely TB	Total
OS1[a]	12/40 (30%)	11/81 (14%)	0/44 (0%)	23/165 (14%)
OS2	12/40 (30%)	12/81 (15%)	3/44 (7%)	27/165 (16%)
OS1 or OS2	17/40 (43%)	19/81 (24%)	3/44 (7%)	39/165 (24%)
IS[b] Xpert MTB/RIF[c]	23/36 (64%)	0/75 (0%)	0/43 (0%)	23/154 (15%)

SCIENTIFIC REPORTS

OPEN

Microbiological diagnosis of pulmonary tuberculosis in children by oral swab polymerase chain reaction

Received: 21 January 2019
Accepted: 15 July 2019
Published online: 25 July 2019

Mark P. Nicol[1,2], Rachel C. Wood[1], Lesley Workman[1], Margaretha Prins[1], Cynthia Whitman[1], Yonas Ghebrekristos[1], Slindile Mbhele[1], Alaina Olson[1], Lisa E. Jones-Engel[3], Heather J. Zar[4] & Gerard A. Cangelosi[3]

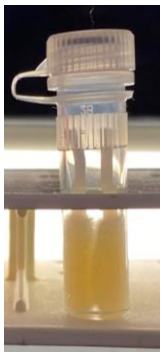
Table 2. Number of positive tests, by TB diagnostic category and test type. [a]OS1, first oral swab PCR; OS2, second oral swab PCR. [b]IS, induced sputum. Of 36 IS culture-positive subjects, 23 (64%) were positive on culture and Xpert, and 13 (36%) were positive on culture only. [c]154 samples were tested by Xpert MTB/RIF.

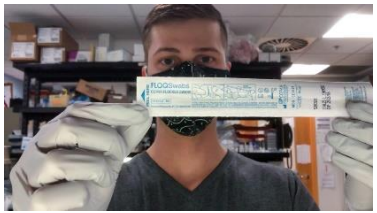
Testing tongue swabs with GeneXpert Ultra MTB/RIF

360 **Table 1. Comparison of Methods 1-3 and manual qPCR method LoDs**

Method	Description	H37Ra LoDs in CFU/swab (95% CI)[a]
Method 1	1 FLOQSwab, SR[b], Xpert Ultra ("single swab SR")	101.7 (64.5 - 144.0)
Method 2	2 FLOQSwabs, SR, Xpert Ultra ("double swab SR")	76.5 (54.2 - 104.1)
Method 3	1 FLOQSwab, boil w/o SR[b], Xpert Ultra ("boil method")	22.3 (15.3 - 34.3)
Manual (Reference)[c]	1 FLOQSwab, Qiagen extraction and EtOH precipitation, manual IS6110 qPCR	53.5 (36.9 – 73.0)

361 [a] LoDs, limits of detection. Contrived samples were tongue swabs from healthy volunteers
362 spiked with dilution series of cultured MTB H37Ra.
363 [b] SR, GeneXpert Sample Reagent
364 [c] Method used in Luabeya et al (2019) and Wood et al (2021)





Grant Whitman

Andama, Whitman, et al (2022). Accuracy of tongue swab testing using Xpert MTB-RIF Ultra for tuberculosis diagnosis. J. Clin Microbiol 60(7):e0042122. PMC9297831.

Testing tongue swabs with GeneXpert Ultra MTB/RIF

	Relative to sputum Xpert	Relative to sputum microbiology
OSA sensitivity	77.8 (64.4-88.0)	73.7 (60.3-84.5)
OSA specificity	100 (97.2-100)	100 (95.8-100)

- Collaboration with R2D2 Network
- N = 183 Ugandan patients
- Double FLOQswabs/SR (method 3)
- Sensitivity somewhat lower than manual method, specificity better
- Signals weak relative to sputum

Semi-quant Xpert results		**Oral swab Xpert Ultra**					
		Negative	Trace	Very low	Low	Medium	Total
Sputum Xpert Ultra*	Negative	127	0	0	0	0	127
	Trace	6	0	0	0	0	6
	Very low	3	0	0	0	0	3
	Low	5	3	0	3	0	11
	Medium	0	3	5	7	0	15
	High	0	1	4	14	2	21
	Total	141	7	9	24	2	183

Clinical analysis by R2D2 Research Network

Andama, Whitman, et al (2022). Accuracy of tongue swab testing using Xpert MTB-RIF Ultra for tuberculosis diagnosis. J. Clin Microbiol 60(7):e0042122. PMC9297831.

Alfred Andama

R2D2 TB Network

Testing tongue swabs with GeneXpert Ultra MTB/RIF

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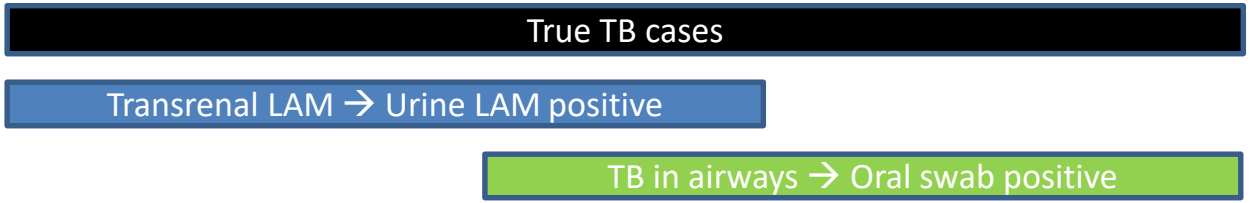
Andama, Whitman, et al (2022).
J. Clin Microbiol
60(7):e0042122. PMC9297831.

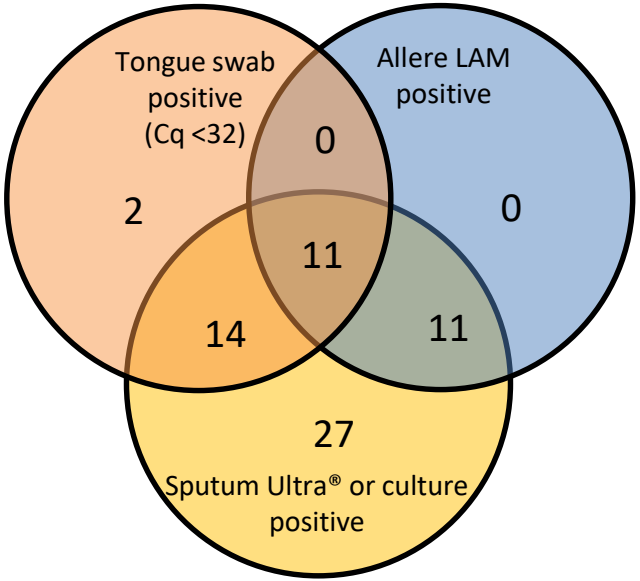
- Collaborators are advised to use Method 3 (single swab/boiling)
  - If boiling isn't feasible, then use Method 2 (double swab/SR)
- Contact us for SOPs and training videos (swabbing and analysis)

Complementary non-sputum detection of TB in HIV-coinfected patients, by using tongue swabs and urine LAM testing

- Sputum is often paucibacillary and/or difficult to collect from AIDS patients
- Tests for mycobacterial lipoarabinomannan (LAM) in urine are viable alternatives but lack sensitivity
- Can a noninvasive LAM + OSA algorithm approach 100% sensitivity?
- BMGF-funded study in KwaZulu Natal, South Africa (with Adrienne Shapiro, Paul Drain, UKZN, and Edendale Hospital, Pietermaritzburg)



- N = 131 patients with possible TB
- 64/131 were TB+ by sputum Ultra or culture
- 120/131 were HIV+
- 130/131 yielded a valid Allere LAM result



Negative in all samples:
65

Sensitivities and specificities relative to sputum testing, at two different Cq cutoffs for OSA positivity

OSA Cq cutoff = 38

	Allere LAM	OSA	Allere LAM or OSA
Sensitivity	22/63 (35%)	42/64 (67%)	45/63 (71%)
Specificity	67/67 (100%)	52/67 (78%)	52/67 (78%)

Sensitivities of Allere LAM vs Allere LAM or OSA: $p < 0.00001$*

Sensitivities of OSA vs Allere LAM or OSA: $p = 0.242$

**Z score, 1-tailed, significant at $p < 0.05$*

OSA Cq cutoff = 32

	Allere LAM	OSA	Allere LAM or OSA
Sensitivity	22/63 (35%)	25/64 (39%)	36/63 (57%)
Specificity	67/67 (100%)	65/67 (97%)	65/67 (97%)

Sensitivities of Allere LAM vs Allere LAM or OSA: $p = 0.006$*

Sensitivities of OSA vs Allere LAM or OSA: $p = 0.021$*

**Z score, 1-tailed, significant at $p < 0.05$*

Oral swab diagnosis of TB

Summary, challenges, and limitations

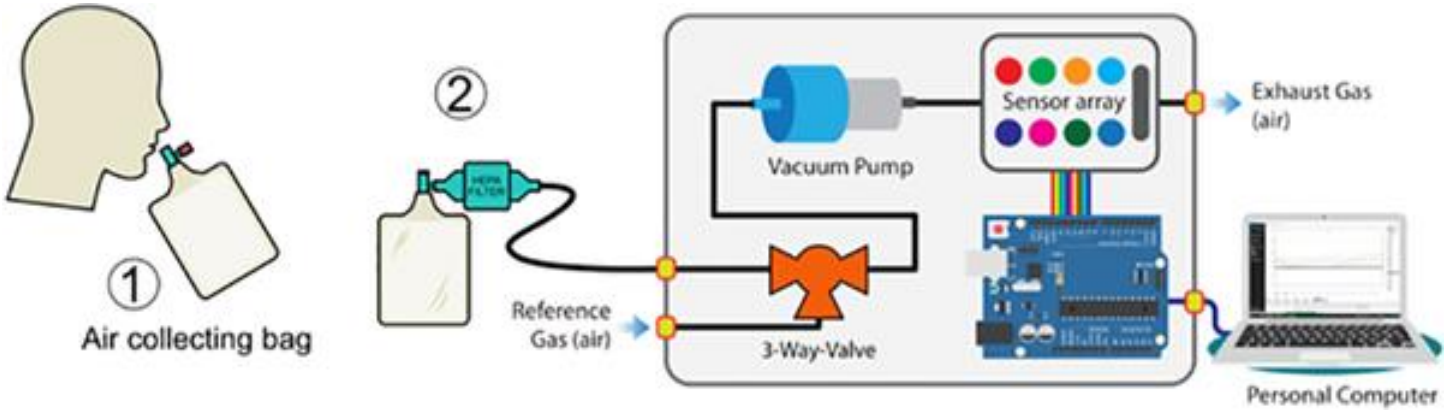
- Tongue swabbing works best in adults
- Easy procedure, universally tolerated, amenable to self-collection
- OSA with GeneXpert Ultra can detect about ~75% of adult pulmonary TB patients
    - Should be considered in settings where sputum collection isn't possible.
- Small-volume sample, not the primary site of infection
- Doesn't (yet) match the sensitivity of sputum testing
    - To improve sensitivity, evaluation of higher-capacity swabs is under way
    - Development of purpose-built OSA POC platforms is under way.

Exhaled breath – volatile organic compounds (VOC)

- Infections change host metabolism, producing distinct combinations of host- and pathogen-derived volatile organic compounds (VOC) in exhaled breath.
- Sample collected using bags, tubes, filters, aerosol concentrators, etc.
- VOCs detected by chemical or physical techniques
  - GC/MS
  - Electronic nose (sensor array)
  - Field asymmetric ion mobility spectrometry (FAIMS)
- Recent systematic review: Saktiawati AMI et al. Diagnosis of tuberculosis through breath test: A systematic review. *EBioMedicine*. 2019;46:202-214.

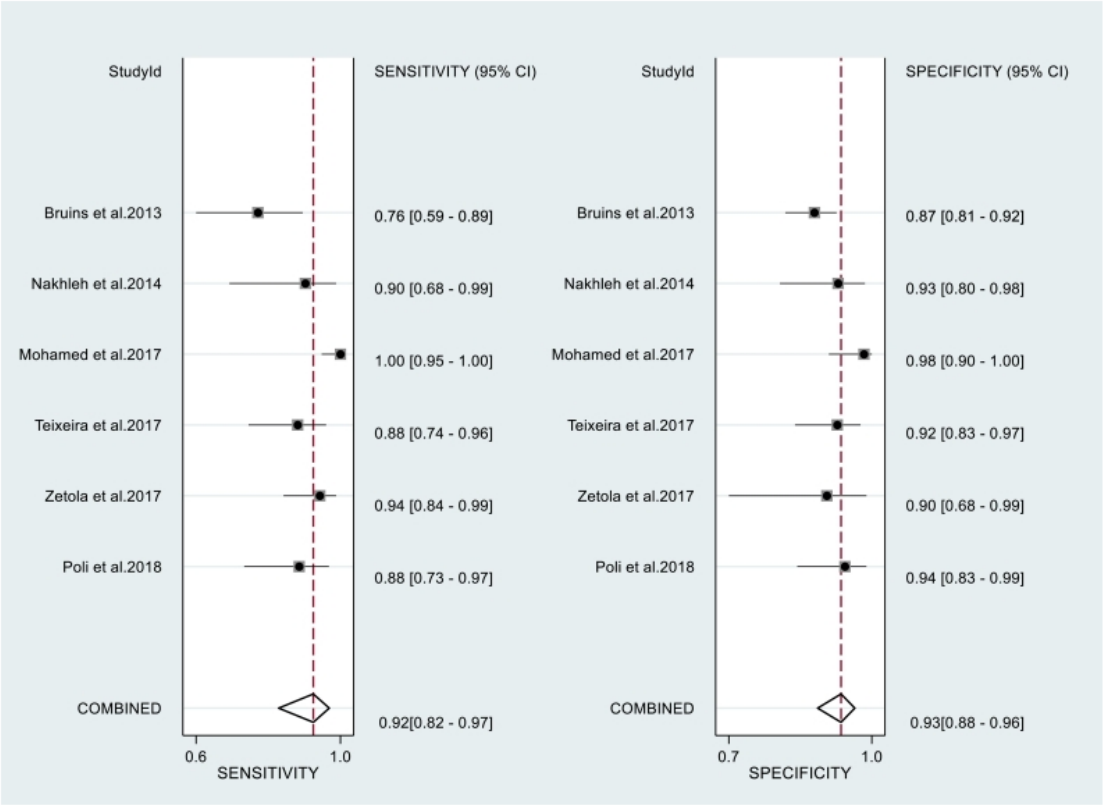
Breath testing

Schematic circuit of the eNose-TB system.



Saktiawati AMI, Triyana K, Wahyuningtias SD, Dwihardiani B, Julian T, et al. (2021) eNose-TB: A trial study protocol of electronic nose for tuberculosis screening in Indonesia. PLOS ONE 16(4): e0249689. https://doi.org/10.1371/journal.pone.0249689

Breath testing (VOC)



Saktiawati AMI et al. Diagnosis of tuberculosis through breath test: A systematic review. *EBioMedicine*. 2019;46:202-214.

Paired forest plots of pooled sensitivity and specificity of electronic nose in diagnosing tuberculosis.

TB diagnosis by breath testing (VOC)

Challenges and limitations

- Most studies conducted to date have focused on extreme sides of the TB disease spectrum
  - Symptomatic, treatment-naïve, smear-positive TB vs. healthy controls with no symptoms
- Novel sample type. It isn't sputum so don't expect 100% sensitivity and specificity relative to sputum

  👍Potential for new types of information
- Diversity in VOC makeup of exhaled breath samples
  - Affected by comorbidities, diet, alcohol, smoking, age, sex, microbiota.
  - Site- and population-specific training analyses needed
- Collection of breath can take time and be logistically challenging
- Sample storage/transport can affect results

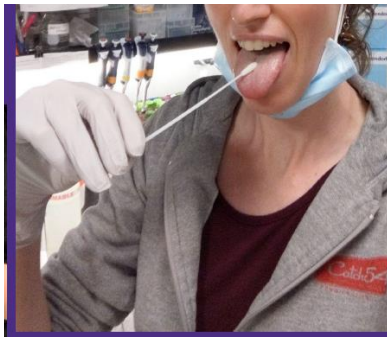
Exhaled breath – MTB DNA

- Face masks can be modified to collected exhaled MTB bacilli and/or DNA
- Detectable by common NAAT's such as GeneXpert MTB/RIF Ultra
- Sensitivities up to 90% have been reported, e.g.
  - Williams CM et al. Exhaled Mycobacterium tuberculosis output and detection of subclinical disease by face-mask sampling: prospective observational studies. Lancet Infect Dis. 2020 May;20(5):607-617.
  - Williams CM et al (2014). Face Mask Sampling for the Detection of *Mycobacterium tuberculosis* in Expelled Aerosols. PLOS ONE 9(8): e104921.

TB diagnosis by breath testing (VOC)

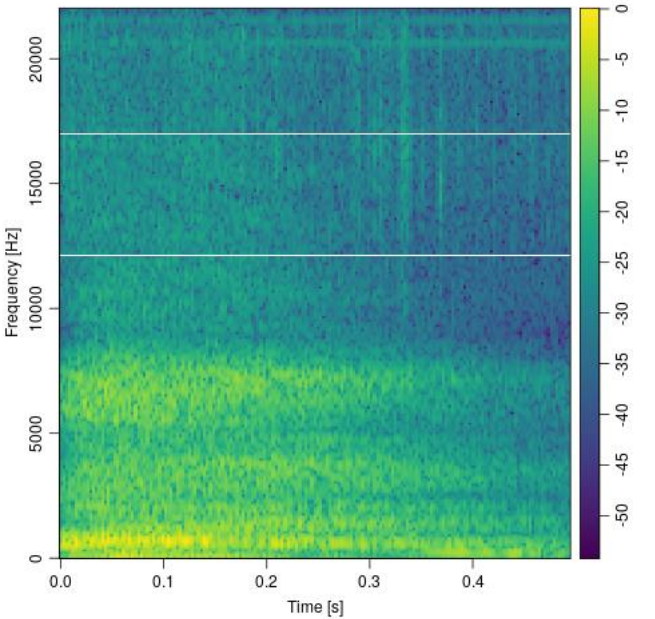
Challenges and limitations

- Sampling method takes time (typically 1 hour wearing mask).
- Masks may be relatively costly
- Might the oral epithelium accomplish the same thing as the gel filter in a mask?



Acoustic monitoring of coughs

- Coughs can be continuously monitored by smart phones and other devices
- Machine learning (combined with appropriate metadata) can assign meaning to cough patterns
- Possible applications
  - Diagnosing TB and distinguishing it from other respiratory diseases
    (what kind of cough is this?)
  - Monitoring treatment and disease progression
    (how often does my patient cough?)
  - Public health surveillance
    (how many different people are coughing here?)



https://www.the-scientist.com/news-opinion/ai-assisted-cough-tracking-could-help-detect-the-next-pandemic--68233

TB diagnosis by acoustic monitoring of coughs

Challenges and limitations

- Biological feasibility remains unproven – work in progress.
  - May be difficult to distinguish a “TB cough” from a “COVID-19 cough”
  - In public settings it remains difficult to discern who is coughing.
  - Applications in patient monitoring and public health surveillance may be more feasible
- Site- and population-specific training analyses needed
  - But the potential for massive data acquisition helps
- Unique ethical, privacy, and user acceptance issues

Why explore alternatives to sputum testing?

Novel samples and analytes

√ New types of information

√ More information

New possibilities for TB control

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