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Oral Rinse as an Alternative Diagnostic Specimen for Detection of Tuberculosis

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To the Editor:

Sputum, the primary specimen for diagnosing pulmonary tuberculosis (TB), is not always reliable. Its collection can also be challenging, especially in young children, the elderly, or severely immunocompromised individuals. The low bacterial loads in these individuals pose challenges for TB detection, often leading to false-negative results. Consequently, alternative nonrespiratory specimens that are noninvasive, safer, and easier to collect, yet maintain diagnostic accuracy, have been explored. Oral/tongue swabs are potential alternatives for TB testing. However, their diagnostic performance, especially by culture, has yielded varied sensitivity and specificity, highlighting the need for further development or exploration of alternative specimens (1–6).

We investigated the diagnostic utility of nonrespiratory specimens, including oral rinse and tongue and cheek swabs. A total of 179 participants (77 from South Africa [SA] and 102 from Brazil) aged ⩾18 years with drug-susceptible TB confirmed by Xpert MTB RIF Ultra assay were recruited at baseline before treatment. Seventeen

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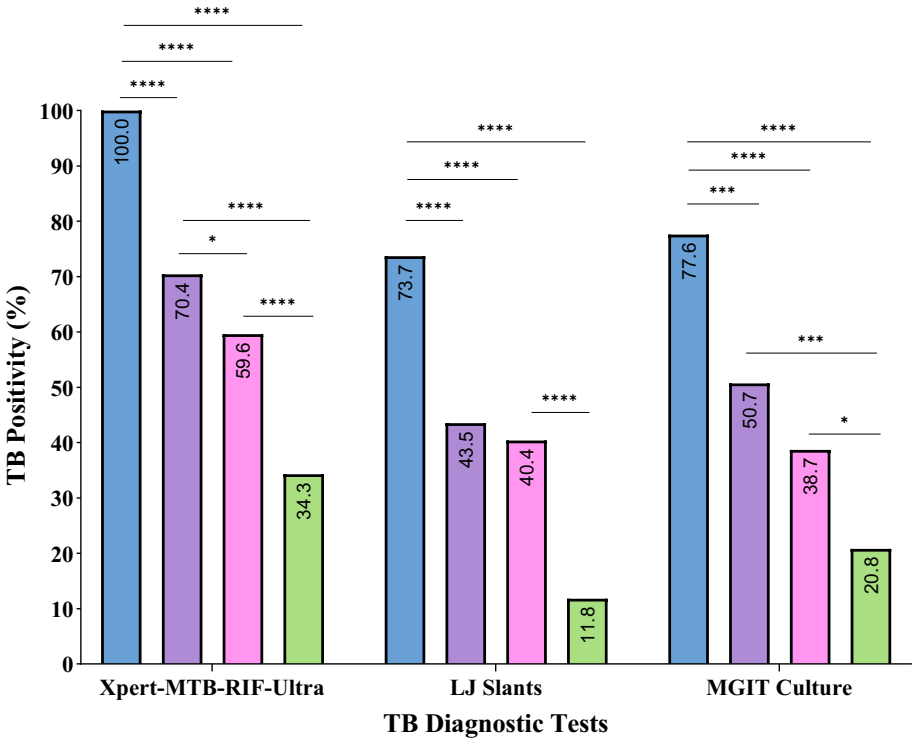

Figure 1. Laboratory detection of tuberculosis (TB) from sputum and nonrespiratory samples in South African (SA) and Brazilian cohorts. Diagnostic performance of the Xpert MTB RIF Ultra assay, Löwenstein-Jensen (LJ) slants, and mycobacteria growth indicator tube (MGIT) cultures in the four sample types: sputum (blue), oral rinse (purple), tongue swab (pink), and cheek swab (green). A total of 179 TB-positive participants were included (SA, $n = 77$; Brazil, $n = 102$). TB-negative control subjects all had negative results on the Xpert MTB RIF Ultra and LJ slant (SA and Brazil; $n = 17$) and MGIT (SA only; $n = 5$). Sample exclusions because of contamination (φ), missing/not collected (δ), or indeterminate (ψ) were as follows: Xpert MTB RIF Ultra: sputum ($n = 179$), oral rinse ($n = 169$; 9^{δ}, 1^{ψ}), tongue swab ($n = 178$; 1^{ψ}), cheek swab ($n = 178$; 1^{ψ}). LJ slant: sputum ($n = 171$; 3^{φ}, 5^{δ}), oral rinse ($n = 168$; 2^{φ}, 9^{δ}), tongue swab ($n = 178$; 1^{φ}), cheek swab ($n = 178$; 1^{φ}). MGIT (SA only): sputum ($n = 76$; 1^{φ}), oral rinse ($n = 71$; 4^{δ}, 2^{φ}), tongue swab ($n = 75$; 2^{φ}), cheek swab ($n = 77$). Sputum showed the highest sensitivity across all methods (Xpert MTB RIF Ultra and LJ slant, $^{****}P < 0.0001$; MGIT, oral rinse, $^{***}P < 0.001$; tongue and cheek swabs, $^{****}P < 0.0001$). Among nonrespiratory samples, oral rinse outperformed tongue and cheek swabs. Key pairwise comparisons: Xpert MTB RIF Ultra: oral rinse versus tongue swab ($^{*}P < 0.05$), versus cheek swab ($^{****}P < 0.0001$). LJ slant: oral rinse versus tongue swab not significant ($^{*}P > 0.05$). MGIT: oral rinse versus cheek swab ($^{****}P < 0.001$), tongue swab versus cheek swab ($^{*}P < 0.05$). Statistical significance was determined using Fisher's exact test with a 95% confidence interval.

individuals (5 from SA and 12 from Brazil presenting with other respiratory conditions but with negative results on Xpert MTB RIF Ultra) served as control subjects. All participants provided written informed consent, and ethics approval was granted by the Human Research Ethics Committee, University of the Witwatersrand, SA (210605B), and CONEP-National Research Ethics Commission, Brazil (38791120.8.0000.5262).

At both sites, oral rinse samples were collected by rinsing the mouth for 1 minute with 2 ml of sterile water. Duplicate oral swabs were collected, one for culture and one for Xpert MTB/RIF Ultra, by brushing a flocked swab eight times on each cheek and another on the tongue. Protocols were translated from Portuguese to English and harmonized for consistency in recruitment, sample collection, and processing across both sites. In the TB-positive group, males predominated in both cohorts (SA, 68.8%; Brazil, 72.5%) with median ages of 35 and 41 years, respectively. In the SA cohort, 54.5% of the participants were people living with HIV, with a median CD4 count of 155 cells/mm^3 compared with 31.4% of participants in the Brazilian cohort with a median CD4 count of 90.5 cells/mm^3. Among control subjects, Brazil ($n = 12$) had 75% males (median age: 48), and SA ($n = 5$) had 80% females (median age, 51 yr). All SA control subjects were people living with HIV (median CD4 count, 257 cells/mm^3) compared with 41.7% in Brazil (median CD4 count, 46 cells/mm^3).

All respiratory and nonrespiratory specimens were decontaminated using *N*-acetyl-L-cysteine-sodium hydroxide (7) within 72 hours from sample collection. Diagnostic performance of the nonrespiratory specimens was compared with *Mycobacterium tuberculosis* positivity in sputum using the Xpert MTB RIF Ultra, which served as the reference standard in this study. In addition, culture including mycobacteria growth indicator tube (MGIT) and Löwenstein-Jensen (LJ) slants assessed mycobacterial viability. Oral rinse samples were missing for 10 participants, and tongue and cheek swabs were missing for 3 participants. Specimens yielding indeterminate or contaminated results in any diagnostic test were excluded before data analysis.

As expected, 100% of the sputum samples had positive test results for *M. tuberculosis* using the Xpert MTB RIF Ultra. With the nonrespiratory samples, oral rinse showed the highest positivity (70.4%), followed by tongue swabs (59.6%) and cheek swabs (34.3%) (Figure 1). Despite overall reduced positivity across all sample types using culture-based methods, oral rinse specimens also yielded

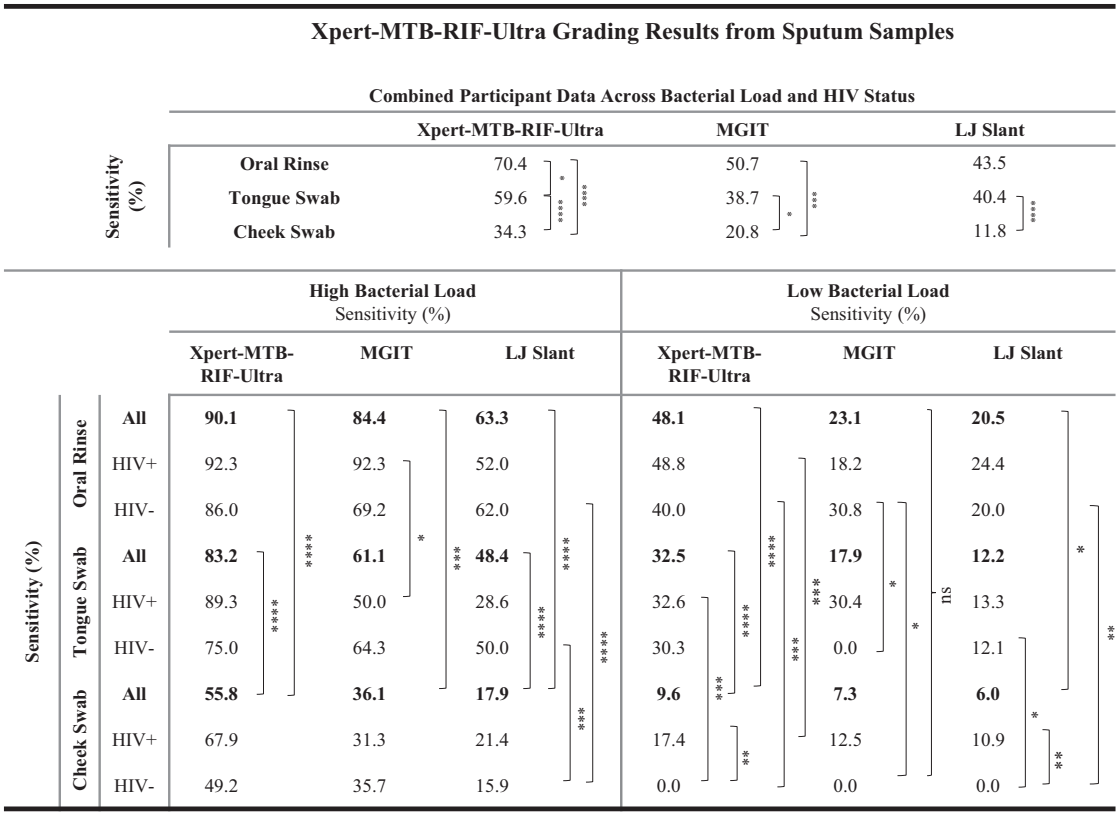

Figure 2. Sensitivity of nonrespiratory samples versus sputum for tuberculosis (TB) diagnosis using the Xpert MTB RIF Ultra assay. The table shows combined data for all participants followed by stratification into high and low mycobacterial load and then further stratification by HIV status, within the high and low bacterial load groups. Sputum was used as the comparator. Sensitivity and specificity values were calculated using the formulae: $\frac{TP}{TP+FN}$; $\frac{TN}{TN+FP}$, respectively. Specificity values are not shown, because all tests and specimen types demonstrated 100% specificity for TB detection. The online tool at https://www.medcalc.org/calc/diagnostic_test.php was used to calculate 95% confidence intervals. Statistical significance was determined using Fisher's exact test with a 95% confidence interval and was based on the TB-positive group and TB-negative group as the reference. Each test was individually assessed for sensitivity and specificity for comparison of these parameters between tests. The groups were classified as follows: true positive (TP) = TB-positive samples from the TB-positive group; false-negative (FN) = TB-negative samples from the TB-positive group; true negative (TN) = TB-negative samples from the TB-negative group; false-positive (FP) = TB-positive samples from the TB-negative group. HIV− = individuals without HIV; HIV+ = individuals with HIV; LJ slants = Löwenstein-Jensen slants; MGIT = mycobacterial growth indicator tube. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

increased positivity on MGIT culture, although this was not statistically significant when compared with tongue swabs, and demonstrated equivalent performance on LJ slants (Figure 1).

To assess the diagnostic utility of the nonrespiratory specimens, their specificity and sensitivity were compared with those of sputum samples using the Xpert MTB RIF Ultra. Oral rinse yielded higher sensitivity than tongue and cheek swabs on the Xpert MTB RIF Ultra (Figure 2). A similar trend was observed for culture-based methods, although the increased sensitivity of the oral rinse did not reach statistical significance compared with tongue swabs (Figure 2). Regardless of bacterial load, sputum and all nonrespiratory specimen types demonstrated 100% specificity across all test conditions (95% confidence interval [CI], 80.5–100 for Xpert MTB RIF Ultra and LJ slants; 47.8–100 for the MGIT assay). We next stratified our samples by bacterial load on the basis of cycle threshold (Ct) values: high or medium Ct values were classified as high bacterial load, and low, very low, or trace Ct values were categorized as low bacterial burden (Figure 2). When benchmarked against sputum, for specimens with high bacterial loads, oral rinse achieved a diagnostic sensitivity of 90.1% (95% CI, 82.1–95.4), with no false-positive results (Figure 2). By high bacterial load, oral rinse and tongue swabs showed comparable sensitivities (90.1% vs. 83.2%, respectively; Figure 2). Tongue swab sensitivity (83.2%; 95% CI, 74.1–90.1) substantially exceeded that of cheek swab (55.8%; 95% CI, 45.2–66.0). Among specimens with low bacterial burden, the sensitivity of TB detection in oral rinse and tongue swabs was similar (48.1%; 95% CI, 36.5–59.7; vs. 32.5%; 95% CI, 22.6–43.7), albeit with reduced sensitivity, consistent with previous findings for oral swabs (8). In this group, tongue swabs outperformed cheek swabs (sensitivity, 9.6%; 95% CI, 4.3–18.1).

With MGIT culture, on high bacterial load specimens, the sensitivity of oral rinse was higher than tongue swabs (84.4% vs. 61.1%, respectively; 95% CI, 67.2–94.7, and 95% CI, 43.5–76.9), an effect also observed in LJ slant culture (63.3% vs. 48.4%; 95% CI, 52.5–73.2, and 95% CI, 38.0–58.9; Figure 2). However, in both cases, these differences were not significant. Stratification of these participants by HIV status showed a significantly higher sensitivity for oral rinse over tongue swabs ($P < 0.05$). For low bacterial load

specimens, culture showed comparable performance for oral rinse and tongue swabs (MGIT, 23.1%; 95% CI, 11.1–39.3; LJ slants, 17.9%; 95% CI, 7.5–33.5) with significantly greater sensitivity of oral rinse on MGIT culture in HIV-negative participants ($P < 0.05$; Figure 2).

Although prior studies have explored oral swabs for TB detection, this study is the first to evaluate a novel oral rinse alongside oral swabs in two geographic settings. Our findings on oral swabs were consistent with previous studies supporting nonrespiratory samples for TB diagnosis (1–6, 8, 9) but demonstrate that a simple oral rinse outperforms tongue swabs for mycobacterial detection using both the Xpert MTB RIF Ultra and select culture-based diagnostic methods.

The oral rinse offers a simple, noninvasive TB diagnostic tool especially useful in resource-limited settings and for individuals unable to produce sputum. Its potential warrants further validation through larger multicenter studies incorporating parallel testing of consecutive specimens, varied rinse formulations and volumes, and optimized DNA extraction and detection methods (8, 10). ■

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Oral Rinse versus Facemask Capture for Nonsputum Diagnosis of Pulmonary Tuberculosis

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To the Editor:

Delays in pulmonary tuberculosis (TB) diagnosis contribute to continued *Mycobacterium tuberculosis* (Mtb) transmission (1). Earlier treatment may reduce TB morbidity, mortality, and socioeconomic burden. TB is primarily diagnosed through passive

A complete list of the Facemask Study Team members may be found before the beginning of the References.

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Author Contributions: S.C.M., T.J.S., and M.H. conceived the idea, raised funds, provided the resources, interpreted the results, and wrote the first draft of the manuscript. S.C.M. provided study oversight and analyzed the data. S.C.M., E.B., and S.V. were responsible for all site-level activities, including recruitment and data collection. B.D.K. assisted in design of the oral rinse experiments. M.S. provided laboratory support and performed the assays. G.T.J., E.W., and S.S. designed and prepared facemasks. All authors had full access to the data; confirm the integrity of the data and its presentation; agree with its interpretation as discussed in the manuscript; and reviewed, revised, and approved the manuscript before submission. S.C.M. had final responsibility for the decision to submit for publication.

Data availability statement: The data that support the findings of this study are available on reasonable request from the corresponding author.

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