

Fast, simple and cheap: method modified from conventional cultivation for tuberculosis diagnosis allows seeding on Löwenstein–Jensen of any swab-embedded pulmonary samples decontaminated with sodium hydroxide

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Background: Few tuberculosis (TB) control programmes in low-income countries have access to culture facilities in their primary care diagnostic centres and this scenario may have worsened with the coronavirus disease 2019 pandemic. Thus the aim was to develop and evaluate a simpler TB test that allows seeding on Löwenstein–Jensen (LJ) medium of several swab-embedded samples decontaminated with sodium hydroxide (NaOH).

Methods: A cotton swab containing each sample was decontaminated in NaOH before being dipped into a slightly acidic solution to neutralize the pH in order to allow the culture to develop on LJ medium. Samples (n=543) from suspected or confirmed pulmonary TB were analysed in two phases: standardization (n=167) and evaluation of the study method (n=376).

Results: The study method showed sensitivity >95% and specificity >93% using Ogawa–Kudoh (OK) and modified Petroff (MP) as standards and was comparable to MP-LJ (p>0.05) and slightly superior to OK (p=0.03) for sputum culture and more comprehensive than the latter for other pulmonary specimens.

Conclusions: This article reports a more comprehensive, simpler and less costly method for diagnosing TB in the laboratory with fewer economic resources and biosafety equipment. Thus a patent application was filed (BR1020190103841).

Keywords: diagnostic techniques and procedures, *Mycobacterium tuberculosis*, pulmonary tuberculosis, sensitivity, specificity, tuberculosis

Introduction

Although recent decades have witnessed increased efforts in the fight to end tuberculosis (TB), fundamental gaps are hampering these efforts, particularly in resource-constrained settings and in settings with a high burden of disease. The World Health Organization (WHO) estimates that close to 54 million TB deaths were averted between 2000 and 2017 due to improved disease prevention and management and service delivery. Nevertheless, TB is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide and the leading

cause of death from a single infectious agent (ranking above human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS)). Accordingly, a total of 1.4 million people died from TB in 2019 (including 208 000 people with HIV). In Brazil, there were an estimated 96 000 new and relapse cases in 2019, corresponding to an incidence of 46/100 000 inhabitants with a mortality rate of approximately 2.2 deaths/100 000 inhabitants. These indicators put Brazil among the 30 high TB burden countries (HBCs) that together accounted for 87% of the world's TB cases in 2019. Of the 30 HBCs, 14 did not reach a treatment

success rate of 85% among all new and relapse TB cases in 2017, including Brazil (71% success), which was the fifth worst in this ranking.¹ Some of these failures are related to delay in TB diagnosis,² which can increase the time of individual exposure to bacilli, hinder TB control, worsen disease and transmission and increase the number of new cases.³

Culturing is the gold standard for diagnosing TB and a highly specific and sensitive detection method that can increase the positivity by up to 30% in cases of negative sputum smear microscopy.⁴ However, samples from the lower respiratory tract have an associated bacterial microbiota that, because of their rapid growth, contaminates the culture medium, preventing mycobacterial recovery.⁵ Sample decontamination using sodium hydroxide (NaOH) is required to eliminate associated microbiota and recover the mycobacteria in the modified Petroff (MP)⁴ and Ogawa–Kudoh (OK) swab culture⁵ methods. The MP method is versatile and recommended for mycobacterial cultivation from sputum, bronchoalveolar lavage and tracheal aspirates in Löwenstein–Jensen (LJ) medium with a pH close to neutral and is the most widely used method.^{4–6} However, MP-LJ requires a suitable centrifuge, biosafety cabinet and specialized personnel to avoid biological risks from contaminated aerosols. In contrast, the Pan American Health Organization (PAHO) recommends use of the OK method for TB diagnosis in areas with limited laboratory capacity and with difficult access to a referral laboratory, but only for sputum samples.⁴ Since it uses 4% NaOH without further neutralization it is considered a drastic method for paucibacillary specimens, which limits its diagnostic application.⁶

Thus this study aimed to develop and evaluate a method for decontaminating and cultivating mycobacterial species and diagnosing TB from sputum and other paucibacillary specimens. Swab-embedded samples decontaminated with NaOH are neutralized before being inoculated on LJ medium, combining the advantages present in conventional cultivation methods for diagnosis of TB. First, a cotton swab from the OK method was used to make decontamination simpler and with a low risk to the handler.⁷ Second, a neutralizer solution, inspired from Darzins method,⁸ was used to minimize the harmful effects of NaOH on viable mycobacteria. Third, the neutralizer solution enabled the use of the LJ medium, as in MP-LJ,⁷ in order to make the cultivation suitable for a greater diversity of samples. This swab culture method, which does not require the use of a centrifuge and biological cabinet, was evaluated to determine its yield in mycobacterial recovery compared with the established methods approved by the WHO.

Methods

Study design

A total of 543 samples from patients with suspected or confirmed pulmonary TB from July 2014 to April 2017 were included in this study, which was carried out in two phases. Phase 1 (n=167) was designed to develop and standardize the mycobacterial culture method, called the OKDPR-TB (in honour of Ogawa, Kudoh, Darzins, Petroff and Rodrigues). Phase 2 (n=376) involved the evaluation of this method at a referral hospital for TB in the state

of Minas Gerais, Brazil, that serves a macroregion of 37 municipalities with around 800 000 Unified Health System users.

Phase 1: standardization and details of the OKDPR-TB method

The set of characteristics for this method in order to produce the lowest contamination rates of mycobacterial cultures were established in this step, with 59 samples tested. Two volumes of 4% NaOH (2.5 mL and 5.0 mL) and two of neutralizer solution (3.0 g of citric acid, 2.5 g of ammonium citrate and 2.0 g of sodium citrate in 100 mL of distilled water, pH 4.5) of 3.0 mL and 4.0 mL were tested. The 4% NaOH at a volume of 5.0 mL and the neutralizer solution at a volume of 4.0 mL were defined as most suitable to avoid contamination and not compromise mycobacterial viability.

Provisional sensitivity and specificity based on test performances of a pilot study at this phase (n=108) were defined using the MP-LJ and OK methods as gold standards. We used only the sensitivity data for the sample calculation because they produced larger sample sizes than the specificity and thus were more appropriate for the definition of both measures of validity in the next phase. According to the pilot study results, the sample size calculated for the next phase was based on a sensitivity range of 80–100% for the OKDPR-TB compared with the two gold standards; an α error of 0.05 and a detection power of 0.8. Minimum sample sizes of 120 sputum samples, 202 other pulmonary specimens and 261 sputum along with other pulmonary specimens were calculated to compare the OKDPR-TB with the MP-LJ method and 110 sputum samples to compare the OKDPR-TB with the OK method.

A cut-off point for the maximum age of the samples was also established in this phase. Samples up to 7 d old were considered suitable for comparisons in the next phase, based on both rates of mycobacterial recovery and contamination during culture.

Phase 2: evaluation of the OKDPR-TB method

Samples from patients with suspected or confirmed pulmonary TB were included. For evaluation of this method, 376 samples were used, assuming a minimum disagreement between the methods. For each sample analysed, a slide was prepared and stained using the Ziehl–Neelsen method to determine the presence of alcohol-resistant acid-fast bacilli (AFB). Then, the samples were prepared and cultured using the MP-LJ, OKDPR-TB and OK methods for sputum samples and the MP-LJ and OKDPR-TB methods for bronchoalveolar lavage (BAL) and tracheal aspirates as follows.

MP-LJ method

Approximately 2 mL of sputum or liquified BAL specimen were mixed in a tube with 2 mL of a 4% NaOH solution and incubated for 15 min at $36\pm 1^\circ\text{C}$ for fluidization decontamination. A volume of 4 mL of water was added, followed by neutralization by titration with an acidic solution until the material turned amber in colour (pH range 6.5–7.2). The tube was centrifuged for 15 min at 3000 g, then 0.1 mL of the neutralized sediment was inoculated into a tube containing LJ culture medium.⁷

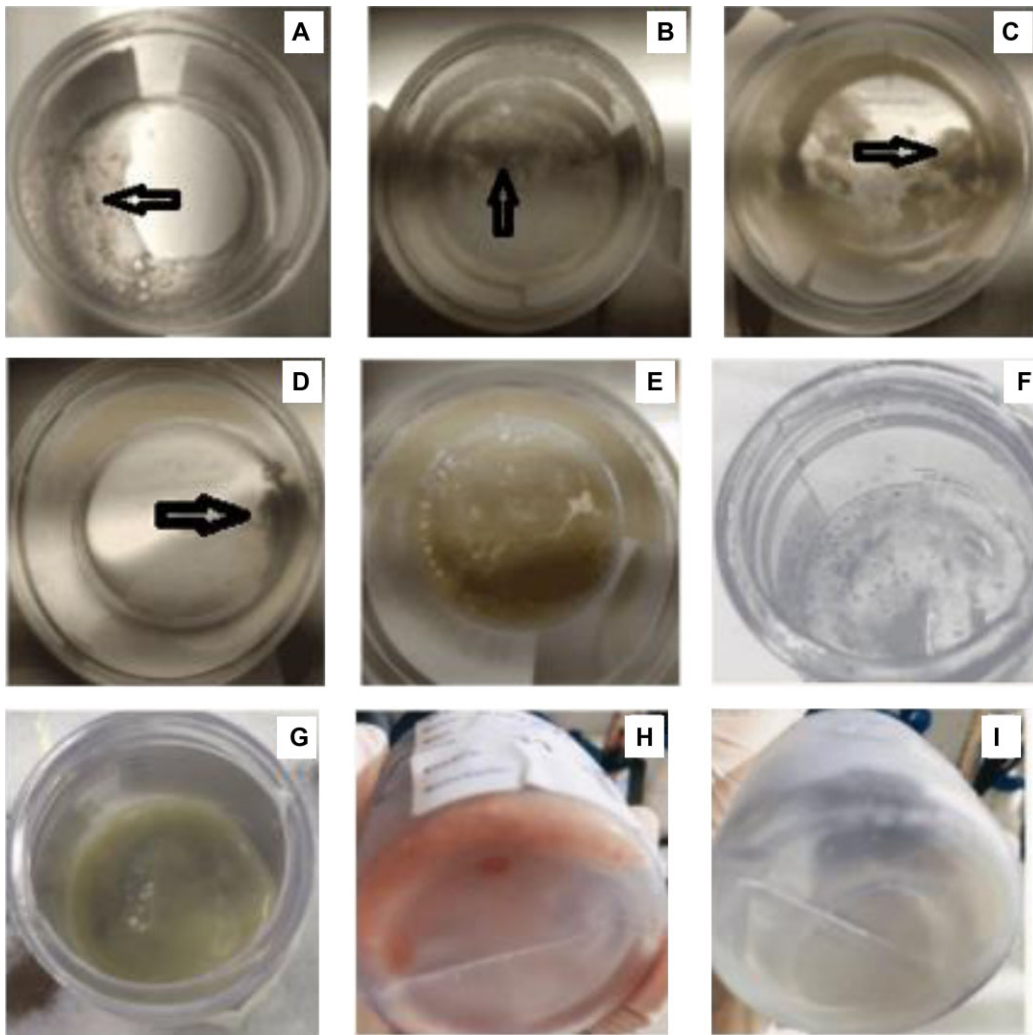


Figure 1. Characteristics of the main study samples showing the preferred points (arrows) for the introduction of the cotton swab: (A) salivary sample with purulent parts, (B) partial salivary and purulent sample, (C) partially purulent sample, (D) salivary sample with a purulent part, (E) purulent sample, (F) saliva sample, (G) bronchial lavage, (H and I) tracheal aspirates. The arrows indicate the most purulent parts that will be picked up by the cotton swab with rotational movements. Swab insertion can be at any point in the samples without arrows, as they are homogeneous. For all samples, rotational movements followed by light pressure on the bottle wall ensures that the most concentrated part of the sample is adhered to the swab and increases the yield of swab culture methods.

OK swab method

The sputum sample (Figure 1) was picked up using a sterile cotton swab, which absorbs approximately 250 μL of the sample. Then the swab impregnated with the sample was immersed in a tube with about 3 mL of sterile 4% NaOH solution, without touching the wall, and remained at rest for 2 min (maximum). After this time, the specimen was directly inoculated into a tube containing Ogawa culture medium (pH 6.4) by streaking the swab over the entire surface of the medium. As there is no neutralization step in the OK method, Ogawa culture medium is slightly acidified to compensate for the inoculum that comes with NaOH.

OKDPR-TB swab method

Either the sputum or BAL sample (Figure 1) was picked up using a sterile cotton swab. The swab impregnated with the sample was

immersed in a tube with about 5 mL of sterile 4% NaOH solution, without touching the wall, and remained at rest for 2 min (maximum) and was then inoculated into a tube containing 4 mL of neutralizer solution, slightly acidic (already described), for 1 min. After this time the specimen was directly inoculated into a tube containing LJ culture medium (pH 7.2 ± 0.2) by streaking the swab over the entire surface of the medium. A summary of the three methods is shown in Figure 2.

Analysis of culture results

The inoculated tubes were incubated at 37°C with the lid loose to dry the inoculum. The tubes were observed in the first 48 h to verify that the inoculum was dry and to determine possible contamination by associated microbiota. Thereafter the tubes were observed daily throughout the first week and weekly for

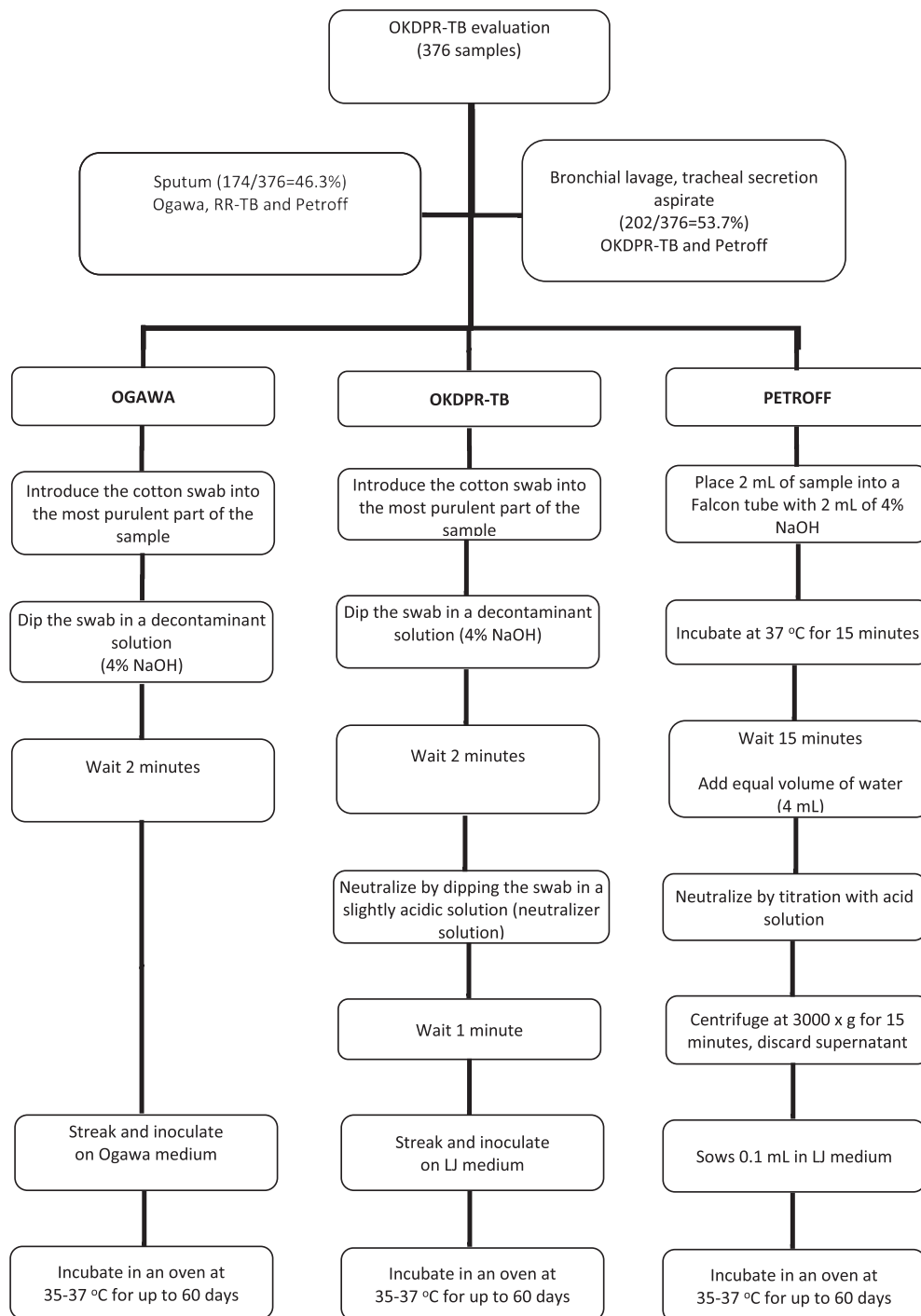


Figure 2. Sample distribution flowchart of the OKDPR-TB method evaluation (n=376) and comparative summary of each method.

60 d. During this period, tubes with growth were removed for confirmation by smear microscopy and tubes without growth were re-incubated. After this period, cultures without growth were recorded as negative.^{5,7} Ziehl-Neelsen and biochemical tests were carried out at the time of detection of visible colonies to confirm *Mycobacterium tuberculosis* complex (MTC). A coding system was adopted to blind the observer when reading the

cultures. Each method received a code (W, Y or X) and the observers could not access this information. Accordingly, an independent observer evaluated the presence of contamination and time and intensity of mycobacterial growth. Figure 3 lists the characteristics of the three methods, with direct (reagents and consumables) and indirect (laboratory structure) cost estimates according to Pinto.⁹

Required items	Methods		
	OKDPR-TB	Ogawa-Kudoh	Modified Petroff
Biological safety chamber	No	No	Yes
Centrifugation	No	No	Yes
Refrigerated centrifuge	No	No	Yes
Shaking	No	No	Yes
LJ culture medium	Yes	No	Yes
Ogawa culture medium	No	Yes	No
Allows sputum processing	Yes	Yes	Yes
Allows tracheal secretion and bronchoalveolar and pericardial lavage processing	Yes	No	Yes
Alkalinization step (decontamination)	Yes	Yes	Yes
pH neutralization step	Yes	No	Yes
Specialized personnel	No	No	Yes
Risk of personnel contamination	Low	Low	High
Total time to perform	3 to 4 min	2 to 3 min	40 to 60 min
Cost	Low	Low	High

Figure 3. Characteristics of the three employed culture methods. Cost estimates: considering direct (reagents and consumables) and indirect (laboratory structure) components.

Statistical analysis

Positivity rates by testing category (smear microscopy and culture) and specimen type (sputum and others) were evaluated by the χ^2 test with a significance level of 0.05.

The McNemar's test using exact binomial probability calculations was used to assess the null hypothesis of homogeneity between two methods performed on paired samples ($p > 0.05$) and the κ index was used to determine the degree of agreement between two tests.

The sensitivity and specificity values and positive and negative predictive values were also determined. Statistical analysis was performed using Epi Info version 7.

Results

We analysed 376 samples (one per person) to validate the OKDPR-TB method, including 174 sputum samples and 202 clinical specimens of BAL and tracheal aspirates (Figure 2).

Sputum smear microscopy

The rate of positive smear microscopy results for the clinical specimens differed ($p < 0.001$) between the sputum (36.8%) and BAL (10.4%) samples.

Culture

The rate of positive sputum cultures was higher than that obtained in BAL, both when the decontamination was performed by the OKDPR-TB ($p < 0.001$) and MP-LJ ($p = 0.001$) methods (Table 1).

The contamination rates were 2.9% and 2.4% for the OKDPR-TB and MP-LJ methods, respectively, in cultures of all specimens ($p > 0.05$), 3.4% for both methods in sputum cultures ($p > 0.05$), 2.5% and 1.5% for the OKDPR-TB and MP-LJ methods, respectively, in cultures of BAL ($p > 0.05$) and 3.4% and 5.7% for the OKDPR-TB and OK methods, respectively, in sputum cultures ($p > 0.05$). The contaminated samples were excluded from further analysis.

Table 1. Performances of OKDPR-TB, MP and OK methods applied in samples of sputum (n=174) and other clinical specimens (n=202) from patients in a public tertiary hospital (Juiz de Fora) between July 2014 and April 2017

Specimens	Total	Query method	Standard method	P-P	N-N	P-N	N-P	p-Values ^a	κ Index	S	Sp ^b	PPV	NPV ^b
All	360	OKDPR-TB	MP	87	257	7	9	0.629	0.88	90.63	97.35	92.55	96.62
AFB positive	80	OKDPR-TB	MP	66	5	5	4	0.753	0.46	94.29	50.00	92.96	55.56
AFB negative/paucibacillary	280	OKDPR-TB	MP	21	252	2	5	0.289	0.84	80.77	99.21	91.30	98.05
Sputum	165	OKDPR-TB	MP	61	95	6	3	0.343	0.88	95.31	94.06	91.04	96.94
Sputum	161	OKDPR-TB	OK	59	94	7	1	0.039	0.89	98.33	93.07	89.39	98.95
Sputum	161	OK	MP	54	95	5	7	0.581	0.84	88.52	95.00	91.53	93.14
AFB-positive sputum	61	OKDPR-TB	MP	52	3	4	2	0.453	0.44	96.30	42.86	92.86	60.00
AFB-positive sputum	60	OKDPR-TB	OK	50	5	5	0	0.031	0.62	100.00	50.00	90.91	100.00
AFB-positive sputum	59	OK	MP	46	4	3	6	0.343	0.38	88.46	57.14	93.88	40.00
AFB-negative/paucibacillary sputum	104	OKDPR-TB	MP	9	92	2	1	0.625	0.84	90.00	97.87	81.82	98.92
AFB-negative/paucibacillary sputum	101	OKDPR-TB	OK	9	89	2	1	0.625	0.84	90.00	97.80	81.82	98.89
AFB-negative/paucibacillary sputum	102	OK	MP	8	91	2	1	0.625	0.82	88.89	97.85	80.00	98.91
BAL	195	OKDPR-TB	MP	26	162	1	6	0.070	0.86	81.25	99.39	96.30	96.43
AFB-positive BAL	19	OKDPR-TB	MP	14	2	1	2	0.625	0.47	87.50	66.67	93.33	50.00
AFB-negative/paucibacillary BAL	176	OKDPR-TB	MP	12	160	0	4	0.062	0.84	75.00	100.00	100.00	97.56

N-N: negative in both methods (true negatives); N-P: negative and positive in query and standard methods, respectively (false negatives); NPV: negative predictive value; P-N: positive and negative in query and standard methods, respectively (false positives); P-P: positive in both methods (true positives); PPV: positive predictive value; S: sensitivity; Sp: specificity.

^ap-Value by the McNemar's test using exact binomial probability calculations (mid-p approach).

^bSome of the low values for specificity and NPVs are due to the high number of positive results among AFB-positive samples.

Validity of OKDPR-TB

The results of the validity analyses are shown in Table 1. Excluding the contaminated samples, 360 were analysed and 103 (28.6%) TB patients were diagnosed by both methods (OKDPR-TB and MP-LJ), 94 (91.2%) were detected with the OKDPR-TB method and 96 (93.2%) were detected with the MP-LJ method, with no significant difference in diagnostic yield between the two methods ($p=0.62$). The OKDPR-TB method had a sensitivity of 95.31%, specificity of 94.06% and a κ index of 0.88 compared with the MP-LJ method.

Separating the samples into sputum samples and BAL, patients were diagnosed as follows. Concerning all sputum samples (n=165), 67 patients were diagnosed by both methods (OKDPR-TB and OK), 66 (98.5%) with the OKDPR-TB method and 60 (89.5%) with the OK method, with a significant difference in diagnostic yield ($p=0.039$). The OKDPR-TB method had a sensitivity of 98.33%, specificity of 93.07% and a κ index of 0.89 compared with the OK method. Accordingly, 70 patients were diagnosed by both methods (OKDPR-TB and MP-LJ), 67 (95.71%) with the OKDPR-TB method and 64 (91.4%) with the MP-LJ method, with no significant difference in diagnostic yield ($p=0.343$). The OKDPR-TB method had a sensitivity of 95.31%, specificity of 94.06% and a κ index of 0.88 compared with the MP-LJ method.

For BAL (n=195), 33 patients were diagnosed by both methods (OKDPR-TB and MP-LJ), 27 (81.8%) with the OKDPR-TB method and 32 (96.9%) with the MP-LJ method, with no significant difference in diagnostic yield ($p=0.07$). The OKDPR-TB method had a sensitivity of 81.25%, specificity of 99.39% and a κ index of 0.86 compared with the MP-LJ method.

There was no significant difference between the three methods ($p>0.05$) for the number of weeks required for MTC colonies to become visible on the culture medium. For all sputum samples, cultures of sputum inoculated with the OKDPR-TB, OK and MP-LJ methods showed growth of 52.08%, 52.08% and 54.16% of visible colonies, respectively, for up to 3 weeks of incubation. Accordingly, cultures of BAL inoculated with the OKDPR-TB and MP-LJ methods showed growth of 43.75% and 50.00% of visible colonies, respectively, for up to 3 weeks of incubation.

Discussion

We developed and evaluated a method for mycobacterial culture, named OKDPR-TB, that allows seeding on LJ of any swab-embedded pulmonary samples decontaminated with NaOH, including paucibacillary samples, which is therefore more comprehensive for several clinical specimens besides sputum. This

method presented a similarity of results, good agreement compared with the gold standards and specificity and sensitivity consistent with that found for the MP-LJ and OK methods in this and other studies.^{10,11} For sputum samples, the OKDPR-TB method even surpassed the other swab reference method for TB diagnosis. The turnaround time for the isolation of MTC was similar among the methods. The percentage of culture contamination was also similar and less than the 10% established by the PAHO.⁴

The MP-LJ method is the most common method. However, due to its high cost and operational difficulties, health services require simpler and more accessible TB tests.^{9,10} The OK method, despite being simpler, less expensive and not requiring a centrifuge, has not been the method of choice in developing countries, perhaps because it is limited to only sputum culture.⁴⁻⁶ The swab-based OKDPR-TB method is an alternative to the OK method, as it expands the cultivation possibilities for several paucibacillary clinical specimens, based on a neutralization step, which allows NaOH-decontaminated swab-embedded clinical samples to be seeded on LJ medium.

The Brazilian Ministry of Health and WHO advocate for a stronger fight against the disease by expanding culture diagnostic methods in primary care,^{11,12} with patient-centred integrated prevention and care and universal access to culturing, which is the most useful tool for diagnosis of pulmonary TB, the choice for monitoring treatment-re-treatment, the criterion for defining bacteriological cure and knowing the resistance profile during treatment.⁴ However, few TB control programmes in low-income countries have access to culture facilities in their primary care diagnostic centres,^{7,11-13} thus new, simpler culture methods such as OK and OKDPR-TB are needed.

The sample decontamination process is a critical point for mycobacterial cultivation in the TB diagnosis. Generally this process uses 4% NaOH for mucolytic activity, but it may be toxic to some mycobacteria, which makes the biological specimen exposure time to this substance crucial for good culture performance.^{1,7} In the OKDPR-TB method, this exposure time is the same as for the OK method, but much shorter than that of the MP-LJ method. Further, the neutralization step of the OKDPR-TB method provides an ideal pH more readily for mycobacterial growth than that of the OK method. In the OK method the neutralization occurs more slowly only after the sample contacts the Ogawa medium.^{6,11} Perhaps this delay partially explains the poorer yield of the OK method compared with the OKDPR-TB method.

As both the OKDPR-TB and OK culture methods do not require sample centrifugation and a small sample volume is absorbed in the swab in comparison with that used in the MP-LJ method, a reduced recovery rate of MTC should be anticipated. Conversely, both swab methods showed similar detection rates with each other and compared with MP-LJ culture. This finding is in agreement with other authors^{10,11,14} and possible explanations have been speculated. A short incubation of 2 min in a high concentration of NaOH is less lethal for mycobacteria than a long incubation at a lower concentration of NaOH, resulting in a lower yield of viable microorganisms when samples are processed with the MP-LJ method, but this is compensated for by its higher sample volume of 2 mL vs 250 μ L.^{10,14} Another hypothesis is that, especially for sputum samples, the concentration that occurs in the MP-LJ method could be offset in the other two methods because

the swab can be directed to the most purulent and more concentrated part of the sample (Figure 1).

Although the direct and indirect costs of the OKDPR-TB method were comparable to those of the OK method in Brazil, the OKDPR-TB showed several advantages. First, there is a slightly higher yield for OKDPR-TB method for sputum samples. Second, the use of LJ and the neutralizer solution, which made it more robust for the isolation of MTC from both BAL and AFB-negative/paucibacillary samples, similar to MP-LJ. Third, there was also evidence that the quality of the colonies was better (more characteristic of mycobacteria) when the samples were seeded in LJ medium, which favoured presumptive visual assessments. This evidence was strengthened with observations from the TB sector of the Ezequiel Dias Foundation (FUNED), the central laboratory of public health in the state of Minas Gerais. This laboratory has verified a higher frequency of both a need for sub-culturing or preparing a smear for AFB, to confirm that it is in fact MTC, and without contamination, when the primary isolation is carried on Ogawa medium. Fourth, the OKDPR-TB method fulfils an assumption, which states that the culture on LJ solid medium remains the gold standard for diagnosis of mycobacterial infection, even using complementary liquid media.^{15,16}

Further, OKDPR-TB, similar to the OK method, requires less infrastructure and, due to less handling of the sample, incurs less risk for the laboratory technician, similar to that found in the smear sputum microscopy procedure, thus requiring less biosafety equipment. Such features of safe culturing of samples and simplicity are especially suitable for countries with both large populations at high risk of TB and a lack of facilities.^{1,4,14} Thus the OK method has been decentralized to municipal referral laboratories or local laboratories in Brazil^{7,10,11} and other Latin American countries¹⁴ in order to reduce the patient's waiting time for test results, since the culture will only be sent to the referral laboratory if there is visualization of colonies. In this case, systematic reference and counterreference flows must be established with the closest referral laboratory to send bacterial isolates for species identification and/or sensitivity testing, enabling expansion of TB testing by culture.^{7,10,11,14}

Swab culture methods are also suitable for the referral laboratory and traditional laboratories with a high sample volume, where it could help to alleviate the workload by eliminating the time-consuming centrifuge step.^{10,11,14} This time savings could be especially important in the coronavirus disease 2019 pandemic era, since cornerstone TB laboratory services have been severely disrupted due to the negative effects of this pandemic, which has competed with TB for laboratories and infrastructure.^{1,17} Accordingly, in 2020, there was a 16% reduction in the notification of new TB cases compared with 2019 in Brazil due to this pandemic situation.¹⁸

This study has some limitations that need to be discussed. We tested the OKDPR-TB method only on samples of pulmonary origin, which is the most epidemiologically important form of TB. However, the validation of the OKDPR-TB method for other extrapulmonary samples, such as urine, faeces, gastric lavage, abscesses and other purulent secretions in general, will make it a more robust method. In addition, we have not yet completed the full validation of the OKDPR-TB method, which will include interassay and interlaboratory assessment of assay repeatability and robustness.

Therefore the OKDPR-TB method showed a more comprehensive, simpler and less costly diagnostic test and these results show this method is a viable alternative to the MP-LJ and OK methods for diagnosing TB in areas with fewer economic resources and biosafety equipment, although it can be used universally in any laboratory or country. Accordingly, due to the innovative features of the OKDPR-TB method, a patent application was recently filed with the National Institute of Industrial Property under number BR 10 2019 010384 1.¹⁹ This has already encouraged three countries (India, China and South Africa) to collaborate with Brazil to expand OKDPR-TB research in those nations. We believe that the dissemination of our promising results will allow health authorities in Brazil and even the WHO, after evaluating the external validity of the present method in different epidemiological realities, to analyse the cost-benefits of expanding or replacing existing methods with the OKDPR-TB.

Author's contributions: RRC created the method and planned and coordinated the laboratory analyses. MRS and ICGL designed the study protocol. MRS performed the statistical analyses and interpretation. RRC and MRS wrote the manuscript. ICGL and CJA critically reviewed the manuscript for intellectual content. All authors read and approved the final manuscript.

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Ethical approval: Procedures followed were in accordance with the ethical standards of the Helsinki Declaration (1964, amended most recently in 2008) of the World Medical Association and the protocol was reviewed and approved by the Brazil's National Research Ethics Committee (no. 816628).

Competing interests: None declared.

Data Availability Statement: The data underlying this article will be shared on reasonable request to the corresponding author.

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