Comparison between Ogawa-Kudoh and modified Petroff techniques for mycobacteria cultivation in the diagnosis of pulmonary tuberculosis

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ABSTRACT

Objective: To compare the performance of the Ogawa-Kudoh method with the modified Petroff technique in diagnosis of pulmonary tuberculosis. Methods: A total of 205 sputum samples from 166 patients with clinical suspicion or under pulmonary tuberculosis follow-up, seen at a public tertiary care hospital, from July 2014 to July 2016 were used. All samples were simultaneously processed using the Ogawa-Kudoh and modified Petroff decontamination methods, according to the recommendations of the Ministry of Health. In the statistical analysis, the McNemar test and the Kappa index were used, respectively, to compare proportions and verify agreement between data. Results: The Ogawa-Kudoh and modified Petroff methods were efficient in mycobacteria detection, with no significant differences in results (p=0.549) and contamination rate of the cultures (p=0.065). The agreement between techniques was considered excellent (Kappa index of 0.877) and Ogawa-Kudoh, as compared to the modified Petroff technique, showed sensitivity of 90.4%, specificity of 96.6%, positive predictive value of 94.3% and negative predictive value of 94.2%. Conclusion: The Ogawa-Kudoh technique proved to be sufficiently sensitive and specific for diagnosis of pulmonary tuberculosis, and, therefore, suitable for routine laboratory application. Since it is simple, low-cost and has less technical requirements for biosafety and professional training, Ogawa-Kudoh is an alternative for managers and healthcare professionals to promote the expansion of bacteriological diagnostic coverage of pulmonary tuberculosis.

Keywords: Ogawa-Kudoh; Petroff; Tuberculosis, pulmonary/diagnosis; Mycobacterium tuberculosis; Sputum; Culture

RESUMO

Objetivo: Comparar o desempenho do método de Ogawa-Kudoh ao de Petroff modificado no diagnóstico da tuberculose pulmonar. Métodos: Utilizaram-se 205 amostras de esarro de 166 pacientes com suspeita clínica ou controle de tuberculose pulmonar atendidos em um hospital público terciário, entre os meses de julho de 2014 a julho de 2016. Todas as amostras foram processadas simultaneamente pelos métodos de descontaminação Ogawa-Kudoh e Petroff
modified, following the guidelines of the Ministério da Saúde. In the analysis, we employed the McNemar test for comparison of proportions, and the Kappa index to verify the concordance between the results. **Results:** The methods of Ogawa-Kudoh and Petroff modification demonstrated efficiency in the detection of mycobacterial infections, with sensitivity levels of approximately 90%. The Kudoh method showed 90.4% sensitivity, 96.6% specificity, and 94.8% positive predictive value. **Conclusion:** The method of Ogawa-Kudoh was demonstrated as efficient, cost-effective, and requiring less technical expertise.

**INTRODUCTION**

The tuberculosis (TB) epidemic is larger than previously announced, with an estimated 10.4 million new cases worldwide in 2015, which represents a 1.4% increase from 2014. Brazil ranks 16th in terms of incidence (41.0/100 thousand inhabitants) and 20th in terms of absolute numbers (81,137 cases reported in 2015), making it essential to improve diagnostic coverage of TB.

In order to meet the goals proposed by the Global End Tuberculosis Strategy (2) report by the WHO (a 95.0% reduction in the absolute number of deaths by TB and a 90.0% drop in incidence rate by 2035, compared with data from 2015), it is essential that diagnostic procedures be simplified and made more accessible to healthcare providers.

**OBJECTIVE**

To compare the performance of the Ogawa-Kudoh and modified Petroff methods in sputum samples of patients with tuberculosis, and verify if the Ogawa-Kudoh method is appropriate for the situation and profile of the disease.
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As to statistical analyses, the parameters of sensitivity, specificity, positive predictive value, and negative predictive value of the Ogawa-Kudoh method were determined, and compared to the modified Petroff method. The McNemar test was used to compare agreement of methods, with a fixed significance level set at 0.05. To verify the agreements among data, the Kappa index was calculated, interpreted according to the Sim and Wright revision. The degree of contamination of the culture media was also assessed with both techniques. The calculations, including the descriptive statistical analysis, were made using the Statistical Package for the Social Sciences (SPSS) software, version 14.0.

RESULTS
This study analyzed 205 samples of sputum from 166 patients in a situation of TB diagnosis or follow-up, processed simultaneously by the Ogawa-Kudoh and modified Petroff decontamination methods. The results of the cultures are detailed on table 1, using the value of p = 0.549 to compare agreement of the methods, and of p = 0.065 to compare the contamination rates of samples among the tests. The Kappa index was 0.877. Relative to the modified Petroff method, the Ogawa-Kudoh method displayed 90.4% sensitivity, 96.6% specificity, 94.3% positive predictive value, and 94.2% negative predictive value.

Table 1. Sputum cultures with decontaminated samples using the modified Petroff and Ogawa-Kudoh methods

| Culture results                  | Modified Petroff n (%) | Ogawa-Kudoh n (%) | p value |
|----------------------------------|------------------------|-------------------|---------|
| Isolation of mycobacteria†       | 76 (37.1)              | 71 (34.6)         | 0.549   |
| Culture negative for mycobacteria| 123 (60.0)             | 121 (59.0)        |         |
| Culture contamination            | 6 (2.9)                | 13 (6.4)          | 0.085   |

† McNemar test for paired samples, with a significance level of 0.05. † identification of positive mycobacteria cultures conducted by the Fundação Ezequiel Dias.

DISCUSSION
In this study, the performance of the Ogawa-Kudoh and modified Petroff methods was equivalent, both in terms of diagnostic agreement and contamination rates. Further, the Kappa index indicated a high degree of agreement between these methods. Ogawa-Kudoh showed high sensitivity, specificity, and agreement relative to the modified Petroff method, corroborating data in literature, and fully capable of being an important alternative for the diagnosis of TB.
Since clinical laboratories play a significant role in the follow-up of TB, the possibility of a quick diagnosis, with a simpler and cheaper execution, such as the Ogawa-Kudoh method, favors a series of activities that help in diminishing the propagation of the disease.\(^7,18,19\) Culture remains as the gold standard for diagnosis of TB, despite more advanced techniques available on the market, and the laboratories are crucial to assess quality of samples for a better performance of the test.\(^3,6\)

Implementing the Ogawa-Kudoh method, especially in priority regions and/or those of difficult access, was suggested by other studies, including within the context of different healthcare services.\(^12-17\) Takao et al.,\(^12\) evaluating sputum samples of patients seen at Primary Care Units, determined that the Ogawa-Kudoh method, compared to the modified Petroff method, proved to be efficient to detect *Mycobacterium tuberculosis* and mycobacteria not belonging to the *M. tuberculosis* complex, even in samples with negative bacilloscopy. The authors found no statistical differences in contamination rates observed by both methods.

Silva et al.,\(^13\) evaluated a 8-year period of use of the Ogawa-Kudoh method in the routine of a reference laboratory in the Northeastern region of the State of Paraná, and obtained results that allowed concluding the technique is an excellent tool for early diagnosis of pulmonary TB. Some studies from other countries, such as those conducted in Venezuela, Uruguay, and Gambia, also demonstrated satisfactory results and adjustment of the Ogawa-Kudoh method to the needs and infrastructure of these different locations.\(^14-16\)

In other studies, the Ogawa-Kudoh method was also compared with other forms of sample pre-treatment. Palaci et al.,\(^8\) showed 94.8% sensitivity and 99.8% specificity of the Ogawa-Kudoh method based on the cases confirmed by cultures processed with N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) and inoculated in Löwenstein Jensen medium. Further, the culture by Ogawa-Kudoh contributed significantly to diagnosis of pulmonary TB in four different regions of Brazil (São Paulo, Espírito Santo, Rio Grande do Sul and Mato Grosso do Sul).\(^8\) Oliveira et al.,\(^17\) obtained results that allowed them to conclude that Ogawa-Kudoh could replace the method using sodium lauryl sulfate/Löwenstein Jensen with no losses in the search for cases in the National Program for Control of Tuberculosis in the State of Rondônia, MT, Brazil.

As to the medium used, it is currently sold ready for use in Brazil, and is even furnished by the Ministry of Health, which recommends its use as an attempt to decentralize the diagnosis of TB and have more lung sample cultures, favoring the diagnosis and consequently, the follow-up of the disease. Furthermore, smaller laboratories, with less sophisticated structures, are able to perform this test — which would not be possible by centrifugation.\(^19\)

The Ogawa-Kudoh method is known, but not used as it could be. Some studies showed its feasibility, including in reference regions, can contribute towards its greater use. Verification of its reproducibility and efficacy in different regions, in distinct contexts of types and levels of healthcare service or population, is of paramount importance to consolidate the technique. By doing so, managers will have more evidence and support to make decisions on its implementation. There are published studies comparing the techniques, but still no conclusive discussion. For this reason, and considering the importance of TB in the context of public health worldwide, the authors deemed this article innovative.

The organization in the *Zona da Mata* region of Minas Gerais is a reference center in the treatment of TB, representing an important site of study of this disease. Additionally, these pieces of information are relevant and strategic for managers, healthcare professionals, and specialists in the subject to support their decisions on a decontamination method. The aim is to reach greater laboratory coverage of pulmonary TB, and subsidize the decision of the Ministry of Health to extend the use of this method.

## CONCLUSION

The Ogawa-Kudoh method was efficient to detect mycobacteria when compared to the modified Petroff method, and no significant discrepancies were observed when comparing test results and feasibility of the isolates (absence of contamination). The agreement between techniques was considered excellent. The findings of this study are applicable to the needs of patients in the region, and can be extrapolated to other patient populations of different regions.

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## REFERENCES

1. World Health Organization (WHO). Global tuberculosis report 2016. Geneva: WHO, 2016.
2. Uplekar M, Weil D, Lonroth K, Jaramillo E, Lienhardt C, Dias HM, Falzon D, Floyd K, Gargioni G, Getahun H, Gilpin C, Glaziou P, Grzemska M, Mirzayev F, Nakatani H, Raviglione M, for WHO’s Global TB Programme. WHO’s new end TB strategy. Lancet. 2015;385(9979):1799-801.
3. Sulis G, Centis R, Sotgiu G, D’Ambrosio L, Pontali E, Spavoglia A, et al. Recent developments in the diagnosis and management of tuberculosis. NPJ Prim Care Respir Med. 2016;26:16078. Review.
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4. Conde MB, Melo FA, Marques AM, Cardoso NC, Pinheiro VG, Dalcin Pde T, Machado Junior A, Lemos AC, Netto AR, Durovri B, Sant’Anna CC, Lima D, Capone D, Barreira D, Matos ED, Mello FC, David FC, Marsico G, Afuine JB, Silva JR, Jamal LF, Telles MA, Hirata MH, Dalcolmo MP, Rabahi MF, Cailleaux-Cesn M, Palaci M, Morrone N, Guerra RL, Dietze R, Miranda SS, Cavalcanto SC, Nogueira SA, Nonato TS, Martire T, Galesi VM, Dettoni Vdo V; Committee on Tuberculosis; BTA Guidelines on Tuberculosis Work Group. III Brazilian Thoracic Association Guidelines on Tuberculosis. J Bras Pneumol. 2009;35(10):1018-48. Review.

5. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Manual de recomendações para o controle da tuberculose no Brasil. Brasília (DF): Ministério da Saúde; 2011. p. 284. [Série A. Normas e Manuais Técnicos].

6. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Manual nacional de vigilância laboratorial da tuberculose e outras micobactérias. Brasília (DF): Ministério da Saúde; 2008. p. 436. [Série A. Normas e Manuais Técnicos].

7. Parsons LM, Somoskövi A, Gutierrez C, Lee E, Paramasivan CN, Abimiku A, et al. Laboratory diagnosis of tuberculosis in resource-poor countries: challenges and opportunities. Clin Microbiol Rev. 2011;24(2):314-50. Review.

8. Palaci M, Peres RL, Maia R, Cunha EA, Ribeiro MO, Lecco R, et al. Contribution of the Ogawa-Kudoh swab culture method to the diagnosis of pulmonary tuberculosis in Brazil. Int J Tuberc Lung Dis. 2013;17(6):782-8.

9. Augusto Cj, Carvalho Wda S, Gonçalves AD, Ceccato Md, Miranda SS. Characteristics of tuberculosis in the state of Minas Gerais, Brazil: 2002-2009. J Bras Pneumol. 2013;39(3):357-64.

10. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Sistema Nacional de Vigilância em Saúde: relatório de situação. Minas Gerais. Brasília (DF): Ministério da Saúde, 2005. p. 34. [Série C. Projetos, Programas e Relatórios].

11. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Departamento de Vigilância das Doenças Transmissíveis. Panorama da tuberculose no Brasil. Indicadores epidemiológicos e operacionais. Região Sudeste. Minas Gerais. Brasília (DF): Ministério da Saúde; 2014. p. 92.

12. Takao EK, Nocchi SR, Siqueira VL, Cardoso MA, Peron ML, Callefi KR, et al. Comparação de métodos de cultivo para o diagnóstico laboratorial da tuberculose pulmonar. Acta Sci Health Sci. 2005;27(2):183-8.

13. Silva FS, Castilho AL, Moltempe FG, Fina RZ, Takao EK, Siqueira VL, et al. Use of the Ogawa-Kudoh method to isolate mycobacteria in a tuberculosis reference laboratory in northwestern Paraná, Brazil. Bras J Pharm Sci. 2013;49(3):567-70.

14. Jaspe RC, Rojas YM, Flores LA, Sofia Toro E, Takiff H, de Waard JH. Evaluation of the Kudoh swab method for the culturing of Mycobacterium tuberculosis in rural areas. Trop Med Int Health. 2009;14(4):468-71.

15. Rivas C, Coelho C, Dafond V, Corbo M, Baldijan M. Performance of the Ogawa-Kudoh method for isolation of mycobacteria in a laboratory with large-scale workload. Rev Argent Microbiol. 2010;42(2):87-90.

16. Jobarteh T, Otu J, Gitteh E, Mendy F, Faal-Jawara T, Ofori-Anyinam B, et al. Evaluation of the Kudoh method for mycobacterial culture: Gambia experience. Int J Mycobacteriol. 2016;5 Suppl 1:S166.

17. Oliveira MS, Lima CA, Moura MM. [Ogawa-Kudoh method of analysis and a comparison with the sodium lauryl sulfate Method-Lowenstein-Jensen for the diagnosis of tuberculosis in the state of Rondônia]. Rev Pesquisa Criação. 2011;10(2):127-37. Portuguese.

18. Procop GW. Laboratory Diagnosis and Susceptibility Testing for Mycobacterium tuberculosis. Microbiol Spectr. 2016;4(6). Review. doi: 10.1128/microbiolspec.TNM7-0022-2016.

19. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Tuberculose – 2015: Detectar, tratar e curar: desafios e estratégias brasileiras frente à tuberculose. Boletim Epidemiológico. 2015;46(9):1-19.