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SENSITIVITY AND SPECIFICITY OF MICROSCOPIC EXAMINATION OF ACID FAST BACILLUS USING DIRECT AND CENTRIFUGATION METHOD IN PULMONARY TUBERCULOSIS PATIENT IN WORK AREA OF PUSKESMAS KABILA

Cicin Triska Sawali[1], Syam S. Kumaji[2], Torajasa Achamar[3]
Universitas Bina Mandiri Gorontalo[1], Universitas Negeri Gorontalo[2], Rumah Sakit Bumi Panua[3]
Email : cintriska23@gmail.com, syamkumaji@gmail.com, torajasa@gmail.com

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ABSTRACT
Tuberculosis (TB) remains a major public health problem worldwide, particularly in developing countries. Microscopic examination of Acid-Fast Bacilli (AFB) is commonly used for pulmonary tuberculosis diagnosis because it is simple, rapid, and cost-effective. However, the sensitivity of direct smear microscopy is often limited by low bacillary concentrations in sputum specimens. Centrifugation has been proposed as a concentration technique to improve AFB detection. This study aimed to compare the sensitivity and specificity of direct smear microscopy and centrifugation methods in detecting AFB among pulmonary tuberculosis patients. A diagnostic test study was conducted in the working area of Kabila Public Health Center. A total of 23 pulmonary tuberculosis patients were selected using purposive sampling. Sputum specimens from each participant were examined using both direct and centrifugation methods. Diagnostic performance was evaluated by calculating sensitivity and specificity values using reference standard data. The majority of respondents were aged 51–60 years (39.1%), male (82.6%), and had a senior high school education (39.1%). The direct microscopy method showed a sensitivity of 86% and a specificity of 100%, whereas the centrifugation method demonstrated a sensitivity of 95% and a specificity of 100%. The centrifugation method exhibited higher sensitivity while maintaining the same specificity as the direct method. These findings indicate that centrifugation improves the detection of AFB in sputum specimens and may serve as a useful alternative approach for supporting pulmonary tuberculosis diagnosis in laboratory settings.

INTRODUCTION

Tuberculosis (TB) remains one of the most important infectious diseases worldwide and continues to be a major public health challenge, particularly in developing countries. According to Okada [1], approximately 60% of global tuberculosis cases occur in Asia, making the region the epicenter of the global TB burden. Countries such as Indonesia, India, and China contribute substantially to the total number of reported cases worldwide. Despite significant advances in disease prevention and treatment, tuberculosis continues to cause considerable morbidity and mortality, especially in low- and middle-income countries where access to healthcare resources and diagnostic facilities remains limited.

Indonesia is recognized as one of the countries with the highest tuberculosis burden globally. The large number of TB cases reported annually indicates that tuberculosis remains a major public health concern requiring continuous efforts in prevention, diagnosis, and treatment [2]. Effective tuberculosis control depends heavily on early case detection because delayed diagnosis may prolong disease transmission within the community, increase the severity of illness, and reduce treatment success rates. Therefore, improving the accuracy of diagnostic methods remains an essential component of TB control programs.

Accurate diagnosis of pulmonary tuberculosis is crucial because the disease is highly transmissible through airborne droplets. Early identification of infected individuals allows prompt initiation of treatment and contributes to reducing transmission rates. Among the available diagnostic approaches, sputum smear microscopy for Acid-Fast Bacilli (AFB) remains the most commonly used method, particularly in resource-limited settings. The method is inexpensive, simple to perform, and capable of providing rapid results, making it suitable for routine use in primary healthcare facilities and tuberculosis control programs [3].

Although sputum smear microscopy is widely used, the diagnostic performance of the direct smear method remains a concern. Direct microscopy relies on the visual identification of acid-fast bacilli in stained sputum specimens. However, the sensitivity of this method is relatively limited, particularly when the number of bacilli present in the specimen is low. Steingart et al. [4], reported that conventional smear microscopy may fail to detect a considerable proportion of tuberculosis cases, especially among patients with paucibacillary disease. Similarly, Lam et al. [5], emphasized that microscopic examination has reduced sensitivity in specimens containing low concentrations of *Mycobacterium tuberculosis*, which may result in false-negative findings.

The limitations of direct microscopy become even more apparent among patients with HIV infection, children, and other immunocompromised populations. Quincó et al. [6], reported that the sensitivity of conventional microscopy decreases substantially in specimens with low bacterial concentrations, making diagnosis more challenging. In addition, sputum samples often contain mucus, cellular debris, and other contaminants that can interfere with microscopic observation and hinder the identification of acid-fast bacilli. Variability in laboratory quality and technical procedures may further affect diagnostic accuracy, highlighting the need for quality assurance and improved specimen processing methods [7].

To improve the diagnostic performance of sputum microscopy, several specimen concentration techniques have been developed. One of the most widely studied approaches is centrifugation. This method involves processing sputum specimens prior to staining in order to concentrate the bacilli into a smaller volume. Through centrifugation, microorganisms are separated from mucus and debris, increasing the likelihood of detecting acid-fast bacilli during microscopic examination [4].

Previous studies have demonstrated the advantages of centrifugation over direct smear microscopy. Uddin et al. [8], found that concentrated smear microscopy produced higher positivity rates compared with direct smear examination. Similarly, Harelimana et al. [9], reported that sputum concentration techniques improved the detection of pulmonary tuberculosis cases, particularly in specimens containing relatively low numbers of bacilli. Albert et al. [10], also emphasized the importance of specimen concentration methods in enhancing the recovery of mycobacteria prior to microscopic examination. These findings suggest that concentrating bacilli before staining may significantly improve the sensitivity of AFB detection.

Despite the promising results reported in previous studies, the implementation of centrifugation methods in routine laboratory practice remains limited, especially in primary healthcare facilities. Additional equipment requirements, longer processing times, and operational considerations may affect its feasibility in resource-constrained settings [11]. Furthermore, evidence comparing the sensitivity and specificity of direct and centrifugation methods in local healthcare settings remains relatively limited. Most previous studies have focused on laboratory performance or specific patient populations, while data from community-based healthcare facilities are still scarce.

Given the continued reliance on sputum smear microscopy for tuberculosis diagnosis and the potential benefits of specimen concentration techniques, further evaluation of diagnostic performance is necessary. Comparative studies examining the sensitivity and specificity of direct and centrifugation methods are important to determine whether the increased diagnostic yield of centrifugation justifies its implementation in routine practice.

Therefore, this study aimed to compare the sensitivity and specificity of microscopic Acid-Fast Bacillus examination using direct and centrifugation methods among pulmonary tuberculosis patients. The findings are expected to provide evidence regarding the diagnostic performance of both methods and contribute to the improvement of tuberculosis diagnostic services, particularly in resource-limited healthcare settings.

RESEARCH METHOD

Study Design

This study employed a diagnostic accuracy study design to compare the diagnostic performance of direct smear microscopy and centrifugation methods in detecting Acid-Fast Bacilli (AFB) among pulmonary tuberculosis patients. The diagnostic performance of both methods was evaluated based on sensitivity and specificity values.

Study Setting

The study was conducted in the working area of Kabila Public Health Center, Bone Bolango Regency, Gorontalo Province, Indonesia, in 2021.

Population and Sample

The study population consisted of pulmonary tuberculosis patients registered at Kabila Public Health Center. A total of 23 respondents were selected using a purposive sampling technique based on predetermined inclusion criteria.

Inclusion Criteria

1. Patients diagnosed or suspected of having pulmonary tuberculosis.
2. Patients who were willing to participate and signed informed consent.
3. Patients who were able to provide sputum specimens for microscopic examination.

Exclusion Criteria

1. Patients with inadequate sputum specimens.
2. Patients who declined participation.
3. Patients with incomplete examination data.

Data Collection Procedures

Data collection began with obtaining informed consent from all participants. Demographic characteristics including age, sex, and educational background were recorded using a structured data collection form.

Sputum specimens were then collected from each participant and examined using two different microscopic techniques:

Direct Smear Microscopy Method

A sputum specimen was directly smeared onto a clean glass slide, stained using the Ziehl–Neelsen staining technique, and examined microscopically for the presence of Acid-Fast Bacilli (AFB).

Centrifugation Method

Sputum specimens were processed using a concentration technique by adding 4% sodium hydroxide (NaOH) solution as a decontaminating agent. The specimens were centrifuged at 3000 rpm for 15 minutes. The sediment obtained after centrifugation was used to prepare a smear, followed by Ziehl–Neelsen staining and microscopic examination for Acid-Fast Bacilli.

Reference Data

The results obtained from the direct smear microscopy and centrifugation methods were compared with the previous laboratory examination data available at Kabila Public Health Center, which served as the reference data for determining diagnostic performance.

Data Analysis

Data were analyzed descriptively using frequency distributions and percentages to describe respondents' characteristics, including age, sex, and educational background.

Diagnostic performance was assessed by constructing a 2 × 2 contingency table for each examination method. The values of True Positive (TP), False Positive (FP), True Negative (TN), and False Negative (FN) were identified and used to calculate sensitivity and specificity.

Sensitivity was calculated using the following formula:

$$\text{Sensitivity} = TP / (TP + FN) \times 100\%$$

Specificity was calculated using the following formula:

$$\text{Specificity} = TN / (TN + FP) \times 100\%$$

The sensitivity and specificity values obtained were used to compare the diagnostic performance of the direct smear microscopy and centrifugation methods in detecting Acid-Fast Bacilli among pulmonary tuberculosis patients.

Ethical Considerations

Participation in this study was voluntary. All respondents received an explanation regarding the objectives and procedures of the study and provided written informed consent prior to participation. The confidentiality of respondents' information was maintained throughout the study.

RESEARCH RESULT

Respondent Characteristics

Age Distribution

Table 1 presents the age distribution of the respondents. Of the 23 pulmonary tuberculosis patients included in this study, the majority were aged 51–60 years (39.1%), followed by those aged 36–50 years (34.8%). Respondents aged 21–35 years and those aged above 60 years each accounted for 13.0% of the study population.

Table 1. Distribution of Respondents by Age

Age Group (Years)	Frequency (n)	Percentage (%)
21–35	3	13.0
36–50	8	34.8
51–60	9	39.1

>60	3	13.0
Total	23	100.0

Gender Distribution

The distribution of respondents based on gender is presented in Table 2. Most respondents were male, accounting for 82.6% of the total sample, while females represented 17.4%.

Table 2. Distribution of Respondents by Gender

Gender	Frequency (n)	Percentage (%)
Male	19	82.6
Female	4	17.4
Total	23	100.0

Educational Background

Table 3 shows the educational background of the respondents. Most respondents had completed senior high school education (52.2%), followed by junior high school (34.8%) and elementary school (13.0%).

Table 3. Distribution of Respondents by Educational Background

Educational Level	Frequency (n)	Percentage (%)
Elementary School	3	13.0
Junior High School	8	34.8
Senior High School	12	52.2
Total	23	100.0

Comparison of Direct Microscopy and Centrifugation Methods

The results of Acid-Fast Bacilli (AFB) examinations using the direct smear method and centrifugation method were compared with the reference (gold standard) results.

Direct Method

Table 4 shows the comparison between the direct smear microscopy method and the reference results. Of the 23 samples examined, 19 were identified as true positives, one as a true negative, and three as false negatives. No false-positive results were observed.

Table 4. Diagnostic Performance of Direct Microscopy

Direct Microscopy	TB Positive	TB Negative	Total
Positive	19	0	19
Negative	3	1	4
Total	22	1	23

Based on these findings, the direct microscopy method yielded a sensitivity of 86% and a specificity of 100%.

Centrifugation Method

Table 5 presents the comparison between the centrifugation method and the reference results. A total of 21 samples were classified as true positives, one as a true negative, and one as a false negative. No false-positive results were found.

Table 5. Diagnostic Performance of the Centrifugation Method

Centrifugation Method	TB Positive	TB Negative	Total
Positive	21	0	21
Negative	1	1	2
Total	22	1	23

The centrifugation method demonstrated a sensitivity of 95% and a specificity of 100%.

Sensitivity and Specificity Analysis

The sensitivity and specificity values of both microscopic examination methods are summarized in Table 6.

Table 6. Comparison of Sensitivity and Specificity Between Direct and Centrifugation Methods

Examination Method	Sensitivity (%)	Specificity (%)
Direct Microscopy	86	100
Centrifugation	95	100

The results indicate that the centrifugation method exhibited higher sensitivity (95%) than the direct microscopy method (86%). However, both methods demonstrated identical specificity values of 100%, indicating excellent ability to correctly identify non-tuberculosis cases.

DISCUSSION

This study aimed to compare the diagnostic performance of direct smear microscopy and centrifugation methods in detecting Acid-Fast Bacilli (AFB) among pulmonary tuberculosis patients. The findings revealed that the centrifugation method demonstrated a higher sensitivity (95%) than the direct smear method (86%), while both methods showed the same specificity value (100%). These results indicate that the centrifugation method has a superior ability to identify true tuberculosis-positive cases without reducing its capacity to correctly identify non-tuberculosis cases.

Tuberculosis remains one of the most important infectious diseases worldwide and continues to be a major public health concern, particularly in developing countries where access to advanced diagnostic technologies is often limited. Although several diagnostic methods such as culture, molecular testing, and immunological assays have been developed, sputum smear microscopy remains the most widely used diagnostic approach in primary healthcare facilities because of its simplicity, affordability, and rapid turnaround time [3]. Consequently, efforts to improve the diagnostic performance of smear microscopy are essential for enhancing tuberculosis case detection, particularly in resource-limited settings.

The present study found that the centrifugation method yielded a higher sensitivity than direct microscopy. This finding suggests that centrifugation improves the likelihood of detecting Mycobacterium tuberculosis in sputum specimens. The higher sensitivity observed may be explained by the concentration process that occurs during centrifugation. Through centrifugal force, bacilli become concentrated in the sediment at the bottom of the tube, increasing the probability of their detection during microscopic examination. In contrast, direct smear microscopy relies on a small volume of untreated sputum, which may not adequately represent the actual bacterial concentration present in the specimen.

The findings of this study are consistent with those reported by Apers et al. [12], who demonstrated that concentration techniques improved the detection of acid-fast bacilli compared with direct microscopy. Their study showed that concentrated specimens produced a higher detection rate because bacilli were more easily visualized after processing. Similarly, Steingart et al. [4], in a systematic review of sputum processing methods, concluded that concentration techniques generally increased the sensitivity of smear microscopy by improving bacillary recovery from sputum samples. The review emphasized that concentration procedures may enhance the diagnostic yield of microscopy, especially among patients with low bacillary loads.

The superiority of centrifugation observed in the present study is also supported by findings reported by Anagaw et al. [13], who found that concentration techniques significantly improved the detection of AFB in sputum specimens. Their study demonstrated that concentrating sputum samples before staining increased the likelihood of identifying positive cases that might otherwise be missed by direct smear examination. Likewise, Uddin et al. [8], reported that concentrated smear microscopy achieved higher sensitivity than direct smear microscopy, reinforcing the value of sputum processing in tuberculosis diagnosis.

Another important factor contributing to the improved sensitivity of centrifugation is the reduction of interfering substances within sputum specimens. Sputum samples frequently contain mucus, epithelial cells, inflammatory debris, and other contaminants that can obscure bacilli during microscopic observation. During centrifugation and specimen processing, these interfering materials are reduced, resulting in cleaner microscopic fields. Consequently, acid-fast bacilli become more visible, enabling laboratory personnel to identify them more accurately. This improvement in specimen quality is particularly beneficial when examining paucibacillary samples, in which the number of bacilli is relatively low.

The use of decontamination procedures before centrifugation may further contribute to improved diagnostic performance. The addition of sodium hydroxide (NaOH) facilitates sputum digestion and liquefaction, allowing bacilli to be released from mucus and cellular material. As a result, microorganisms become concentrated within the sediment fraction without significant structural damage to the bacilli. Improved specimen homogenization increases the probability of detecting AFB microscopically and reduces the occurrence of false-negative results. This mechanism may explain why the centrifugation method identified more true-positive cases than the direct method in the present study.

Despite its lower sensitivity, direct smear microscopy remains an important diagnostic tool in tuberculosis control programs. In this study, the direct method achieved a sensitivity of 86% and a specificity of 100%, indicating satisfactory diagnostic performance. The method offers several practical advantages, including simplicity, low cost, minimal equipment requirements, and rapid processing time. These characteristics make direct microscopy particularly suitable for primary healthcare facilities with limited laboratory infrastructure. Therefore, although centrifugation provides superior sensitivity, direct smear microscopy continues to play a critical role in routine tuberculosis diagnosis, especially in remote or resource-constrained settings.

An important finding of this study is that both methods demonstrated perfect specificity (100%). This indicates that neither method generated false-positive results among the examined samples. High specificity is essential in tuberculosis diagnosis because false-positive results may lead to unnecessary treatment, increased healthcare costs, and psychological burden for patients. The absence of false-positive findings suggests that both methods were highly effective in correctly identifying individuals who did not have tuberculosis according to the reference standard.

From a public health perspective, improved sensitivity is particularly important because undetected tuberculosis cases contribute to ongoing disease transmission within communities. Patients with false-negative results may continue to spread infection while remaining untreated. Therefore, diagnostic methods capable of identifying a greater proportion of true-positive cases can significantly enhance tuberculosis control efforts. The higher sensitivity achieved by centrifugation in this study suggests that its implementation could improve case detection rates and facilitate earlier treatment initiation.

Regarding respondent characteristics, the majority of participants were aged 51–60 years. This finding may reflect the cumulative effects of prolonged exposure to tuberculosis risk factors and the gradual decline in immune function associated with aging. Previous studies have shown that individuals within older age groups are more susceptible to tuberculosis due to immunosenescence and the presence of comorbid conditions that compromise host defenses. Furthermore, adults in productive age groups often experience extensive social interaction and occupational exposure, increasing opportunities for transmission.

The predominance of male respondents observed in this study is consistent with epidemiological patterns reported in many tuberculosis investigations. Men generally exhibit higher tuberculosis notification rates than women. Several factors may contribute to this pattern, including occupational exposure, smoking behavior, healthcare-seeking practices, and differences in social activities. Although gender alone may not directly determine tuberculosis occurrence, behavioral and environmental factors frequently associated with males can increase exposure to infection.

Most respondents had completed senior high school education. Educational level is often associated with health literacy, awareness of disease symptoms, healthcare utilization, and treatment adherence. Individuals with higher educational attainment may possess better knowledge regarding disease prevention and management. Nevertheless, tuberculosis can affect individuals across all educational levels, particularly when socioeconomic and environmental risk factors are present.

Several limitations should be considered when interpreting the findings of this study. First, the sample size was relatively small, involving only 23 respondents, which may limit the statistical power and generalizability of the results. Second, the study was conducted in a single healthcare facility, potentially restricting the applicability of the findings to other populations and settings. Third, culture-based confirmation, which is widely recognized as the reference standard for tuberculosis diagnosis, was not performed directly within the study. Future investigations involving larger sample sizes, multiple healthcare centers, and comprehensive reference standards are recommended to strengthen the evidence regarding the diagnostic performance of centrifugation methods.

Overall, the findings of this study demonstrate that the centrifugation method provides superior sensitivity compared with direct smear microscopy while maintaining equally high specificity. These results support the potential use of centrifugation as an alternative approach to improve AFB detection in pulmonary tuberculosis patients, particularly in healthcare facilities seeking to enhance diagnostic accuracy without relying on expensive molecular technologies.

CONCLUSION

The centrifugation method demonstrated higher diagnostic sensitivity than the direct smear microscopy method for detecting Acid-Fast Bacilli in pulmonary tuberculosis patients, while both methods showed the same specificity. The concentration process achieved through centrifugation improved the visibility and detection of bacilli, particularly in specimens with low bacterial loads. These findings indicate that the centrifugation method provides better diagnostic performance and can be considered a valuable alternative to direct microscopy for improving tuberculosis case detection in clinical laboratory practice. Future studies involving larger sample sizes and reference standards such as culture or molecular testing are recommended to further validate these findings.

REFERENCES

[1] K. Okada, "Epidemiological situation of tuberculosis in Asia," *Japanese J. Chest Dis.*, vol. 75, no. 5, pp. 495–507, 2016, [Online]. Available: https://www.scopus.com/inward/record.uri?eid=2-s2.0-

84973514571&partnerID=40&md5=c2ac819e25fe083ded2c686c260b5f5e

[2] S. Hansun, "TB CNR Prediction Using H-WEMA: A Five Years Reflection," *Int. J. Adv. Soft Comput. its Appl.*, vol. 12, no. 3, pp. 1–10, 2020, [Online]. Available: https://www.scopus.com/inward/record.uri?eid=2-s2.0-85098576337&partnerID=40&md5=7c857d6272fcc246a8d5fdbeb35c892e

[3] J. C. Palomino and A. Martin, "Challenges Associated with Diagnostics, Drug Resistance, and Pathogenesis of Mycobacterium tuberculosis," in *Human Emerging and Re-emerging Infections Viral and Parasitic Infections: Volume 2*, vol. 2, Laboratory of Microbiology, Department of Biochemistry and Microbiology, Ghent University, Ghent, Belgium: wiley, 2015, pp. 863–876. doi: 10.1002/9781118644843.ch45.

[4] K. R. Steingart *et al.*, "Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review," *Lancet Infect. Dis.*, vol. 6, no. 10, pp. 664–674, 2006, doi: 10.1016/S1473-3099(06)70602-8.

[5] P. K. Lam, A. Catanzaro, S. Perry, and P. A. Lobue, "Diagnosis of pulmonary and extrapulmonary tuberculosis," in *Reichman and Hershfield's Tuberculosis: A Comprehensive, International Approach, Third Edition*, Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of California, San Diego, United States: CRC Press, 2006, pp. 155–181. [Online]. Available: https://www.scopus.com/inward/record.uri?eid=2-s2.0-67649568495&partnerID=40&md5=ed2e85ed96668d587f692d4d537d9072

[6] P. Quincó *et al.*, "Increased sensitivity in diagnosis of tuberculosis in HIV-positive patients through the small-membrane-filter method of microscopy," *J. Clin. Microbiol.*, vol. 51, no. 9, pp. 2921–2925, 2013, doi: 10.1128/JCM.00683-13.

[7] H. Şimşek, I. Ceyhan, G. Tarhan, and U. Güner, "Quality assessment of microscopic examination in tuberculosis diagnostic laboratories: A preliminary study," *Mikrobiyol. Bul.*, vol. 44, no. 4, pp. 561–569, 2010, [Online]. Available: https://www.scopus.com/inward/record.uri?eid=2-s2.0-78649269982&partnerID=40&md5=d11a6514ddb9c5bb859eec47efca4c9a

[8] M. K. M. Uddin *et al.*, "Comparison of direct versus concentrated smear microscopy in detection of pulmonary tuberculosis," *BMC Res. Notes*, vol. 6, no. 1, 2013, doi: 10.1186/1756-0500-6-291.

[9] J. D. Harelimana *et al.*, "Sputum concentration improves diagnosis of pulmonary tuberculosis cases in children at a tertiary care institution in Rwanda," *Rwanda Med. J.*, vol. 70, no. 1, pp. 15–18, 2013, [Online]. Available: https://www.scopus.com/inward/record.uri?eid=2-s2.0-84893040075&partnerID=40&md5=bdbbf98267ff19896614f9099b1659b3

[10] H. Albert *et al.*, "Feasibility of magnetic bead technology for concentration of mycobacteria in sputum prior to fluorescence microscopy," *BMC Infect. Dis.*,

vol. 11, 2011, doi: 10.1186/1471-2334-11-125.

[11] E. Ramos *et al.*, "Optimizing tuberculosis testing for basic laboratories," *Am. J. Trop. Med. Hyg.*, vol. 83, no. 4, pp. 896–901, 2010, doi: 10.4269/ajtmh.2010.09-0566.

[12] L. Apers *et al.*, "A comparison of direct microscopy, the concentration method and the Mycobacteria Growth Indicator Tube for the examination of sputum for acid-fast bacilli," *Int. J. Tuberc. Lung Dis.*, vol. 7, no. 4, pp. 376–381, 2003, [Online]. Available: https://www.scopus.com/inward/record.uri?eid=2-s2.0-0037388936&partnerID=40&md5=d52a5380567e7ade9bbe9e2e37c35a55

[13] B. Anagaw *et al.*, "Improved detection of Acid-fast bacilli in sputum by the bleachconcentration technique at condar university teaching hospital, Northwest Ethiopia," *Ethiop. Med. J.*, vol. 50, no. 4, pp. 349–354, 2012, [Online]. Available: https://www.scopus.com/inward/record.uri?eid=2-s2.0-84876220996&partnerID=40&md5=5b893e76cbca1a6133c9f6aed2a73f6e