Evaluation of a simple loop-mediated isothermal amplification test kit for the diagnosis of tuberculosis

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OBJECTIVE: A new loop-mediated isothermal amplification (LAMP) test kit, including a simple DNA extraction device for the detection of Mycobacterium tuberculosis complex, was developed for commercial use and evaluated for its usefulness in diagnosing tuberculosis (TB).

DESIGN: The LAMP test was performed using untreated and N-acetyl-L-cysteine (NALC) NaOH-treated sputum specimen. The efficiency of the kit was compared with other conventional laboratory examinations, including other nucleic acid amplification (NAA) tests.

RESULTS: The sensitivity of LAMP using raw sputum (direct LAMP) in smear- and culture-positive specimens was 98.2% (95%CI 94.9–99.4), while the sensitivity in smear-negative, culture-positive specimens was 55.6% (95%CI 43.4–68.0). The diagnostic sensitivity of direct LAMP for the diagnosis of individuals with TB was 88.2% (95%CI 81.4–92.7). The sensitivity values of direct LAMP were slightly, but not statistically significantly lower than those of Cobas Amplicor MTB and TRC Rapid MTB, while the sensitivity of the LAMP test using NALC-NaOH treated sputum was significantly lower than other NAA tests (P < 0.05) for smear-negative, culture-positive specimens. The new commercial version of the LAMP kit was easy to handle and yielded results within 1 h of receiving sputum specimens.

CONCLUSIONS: This test is considered a promising diagnostic tool for TB, even for peripheral laboratories with limited equipment, such as those in developing countries.

KEY WORDS: LAMP; tuberculosis; diagnosis

TUBERCULOSIS (TB) remains a major life-threatening disease.1 Direct sputum smear microscopy is the main diagnostic tool for the detection of TB in many developing countries. However, the sensitivity of smear microscopy is relatively low, and this could be a major obstacle to the early diagnosis of TB. The human immunodeficiency virus (HIV) pandemic further reduces its value due to the low yield of acid-fast bacilli (AFB) in sputum specimens from HIV-infected TB patients.2–4

Nucleic acid amplification (NAA) is a powerful diagnostic tool for the detection of TB, with high efficiency. It has been reported that the sensitivity and specificity of NAA is almost equal to that of culture in smear-positive specimens.5–11 Even in developing countries, NAA can deliver high performance in detecting the Mycobacterium tuberculosis complex (MTC).12–14

Although NAA is widely utilised in many advanced countries, it is still not routinely applicable in developing countries due to its high cost, complicated procedures, insufficient laboratory facilities and shortage of skilled technologists.15–17

Loop-mediated isothermal amplification (LAMP, Eiken Chemical Co Ltd, Tokyo, Japan) is a simple NAA method that does not require expensive devices or detection systems.18–20 Boehme et al. recently reported that the LAMP method showed 97.7% sensitivity with smear-positive sputum specimens, and 48.8% with smear-negative, culture-positive specimens, with a short amplification time.21 Eiken Chemical has modified the kit used in that study, and has developed a new simple, contamination-resistant kit for the diagnosis of TB. This new LAMP kit was evaluated to diagnose TB in the present study.
MATERIALS AND METHODS

Patients and clinical specimens

This study was conducted at the Double-Barred Cross Hospital and Tokyo Hospital in Japan. All participants were suspected to be positive for TB, with typical symptoms and/or chest X-ray findings. A total of 170 patients (TB and non-TB) were enrolled in the study. The patients (TB suspects) were asked to submit two sputum specimens (at least 2 ml) on two consecutive mornings; 340 sputum specimens were collected from June to December 2009. Patients were finally diagnosed based on bacteriological, histopathological and/or clinical findings, such as clinical improvement after anti-tuberculosis chemotherapy. *Mycobacterium* spp. other than *M. tuberculosis* (MOTT) infections were diagnosed using Japanese Anti-Tuberculosis Association criteria.22

The present study was approved by the Ethics Committee of both hospitals. Subjects were enrolled after obtaining written informed consent. Subjects aged <18 years, and those who had been receiving anti-tuberculosis treatment for >48 h, including fluoroquinolones and aminoglycosides 60 days prior to enrolment, were excluded.

AFB smear and culture

Morning sputum specimens were collected from the patients. The quality of each specimen was recorded using the criteria of Miller and Jones.23 Untreated sputum specimens were divided equally into two tubes; one specimen was treated using the standard N-acetyl-L-cysteine (NALC) NaOH digestion method, followed by neutralisation with phosphate buffer (PB; pH 6.8) and centrifugation at 3000 g for 15 min at 4°C. The sediment was re-suspended in 2 ml of PB, and one part was submitted for auramine-O fluorescent smear microscopy. The same re-suspended specimen was cultured with BACTEC Mycobacterial Growth Indicator Tube (MGIT) 960 (Nippon Becton Dickinson Co Ltd, Tokyo, Japan) and 2% Ogawa medium (Kyokuto Pharmaceuticals, Tokyo, Japan) at 37°C for up to 6 and 8 weeks, respectively. All positive cultures were subjected to identification with immunochromatographic (CapiliaTB; TAUNS, Shizuoka, Japan) methods. If MOTT were isolated, identification tests were performed using nucleic acid probes (DDH mycobacterium ‘Kyokuto’; Kyokuto Pharmaceuticals, Japan).

One part of the re-suspended sediment was subjected to the LAMP test and Cobas Amplicor MTB (Roche Diagnostics Systems Inc, Tokyo, Japan) or TRC Rapid MTB (Tosoh Co, Tokyo, Japan) for the detection of MTC. If the NAA tests were not performed immediately, the treated specimen was stored at −20°C until use. Another raw sputum specimen was subjected to direct fluorescent smear microscopy and the LAMP test using approximately 40 μl of the purulent part. The whole process was repeated for the second specimen.

LAMP and other NAA s

A commercial version of the LAMP kit for TB detection was used in the study. The kit was provided by Eiken Chemical and was composed of a DNA extraction device and LAMP reaction tube. Approximately 40 μl of untreated/treated sputum specimen was mixed with the DNA extraction solution (960 μl) by inversion in a proprietary tube (heating tube) and incubated at 90°C for 5 min using a proprietary heat block. After incubation, the DNA was purified through porous material in a closed system (adsorbent tube) called PURE (Procedure for Ultra Rapid Extraction, Eiken Chemical). The extracted DNA in 30 μl of solution was directly added to the LAMP reaction tube containing dried LAMP reaction mix (primers, dNTPs, buffer and Bst DNA polymerase). The primers used in this kit were modified from those previously reported,21 and had high species specificity for MTC (internal data). The LAMP reaction tube was mixed by inversion (five times) and incubated at 67°C for 40 min (Figure). A positive LAMP result was detected by real-time measurement of turbidity and visually using fluorescence under ultra-violet light. The LAMP test was also performed using NALC-NaOH treated specimens.

Cobas Amplicor MTB or TRC Rapid MTB were performed according to the manufacturer’s instructions using respectively 100 and 200 μl of treated sputum specimen. A final volume of respectively 25 and 20 μl was used for each reaction. Tokyo Hospital used TRC Rapid MTB for the first specimen only due to capacity limitations.

Statistical analysis

Analyses were conducted on the sensitivity and specificity of the LAMP test for active TB compared to other NAA tests; the difference in these parameters was tested using the χ² method. A linear regression analysis with smear positivity was also performed. Statistical analysis was performed using JMP 6.0.3 (SAS Institute, Cary, NC, USA). P < 0.05 was considered statistically significant.

RESULTS

Patient diagnosis

A total of 10 patients suspected of having TB were excluded, as one patient died on the first day and the remaining nine did not conform to the enrolment criteria. The final analyses were performed for 320 specimens from 160 patients. No specimen was excluded from the analyses. A total of 127 patients were diagnosed with active TB, and 18 had MOTT infections. Among the 127 active TB patients, 119 were MTC culture-positive, while the remaining eight
were culture-negative. Three culture-negative TB cases were smear-positive and all were NAA-positive, while five were negative for all bacterial examinations, but were diagnosed using chest X-ray findings and antimicrobial treatment response. Non-mycobacterial diseases were found in 15 patients: bronchiectasis with bacterial infection \((n = 3)\), bacterial pneumonia \((n = 2)\), lung cancer \((n = 2)\), bronchial asthma \((n = 2)\), sarcoidosis \((n = 1)\), pulmonary aspergillosis \((n = 1)\) and lung abscess \((n = 1)\).

**AFB smear and culture**

Among 320 sputum samples from 160 TB suspects, respectively 188 (58.8%) and 201 (62.8%) specimens were direct and indirect (concentrated) smear-positive. The quality of 179 (55.6%) of the 320 sputum specimens was M1 or M2, while 66 (20.6%) were P3.* A total of 115 (71.9%) suspects were smear-positive and 45 (28.1%) were negative. Of 254 sputum specimens from active TB cases, respectively 176 (69.3%) and 188 (74.0%) were positive for direct and indirect smear examination.

Solid cultures were positive for 214 (66.9%) specimens, while MGIT cultures were positive for 236 (73.8%) specimens. The majority of the positive specimens were due to MTC (223 isolates in total), while 16 isolates were *M. avium* or *M. intracellularare*, four isolates were *M. kansasii* and one isolate was *M. xenopi*. Mixed infection (MTC and MOTT) was detected in two specimens.

**LAMP vs. other NAAs**

The results of LAMP using untreated (raw: direct) and treated (digested and concentrated: indirect) sputum specimens are summarised in Table 1. A result was considered positive if either direct or indirect smear examination of the specimen was positive. Sensitivity was calculated using the culture result as the gold standard. As described above, some MOTTs were isolated by culture; they were, however, classified in the culture MTC-negative group. Cases of mixed infection with MTC and MOTT were considered to be culture MTC-positive and TB-positive.

The number of valuable test results for LAMP was 320. Respectively 205 and 198 specimens were positive for direct and indirect LAMP. Similarly, respectively 318 and 244 specimens were tested by Cobas Amplicor MTB and TRC Rapid MTB. Two Cobas Amplicor MTB tests were missed due to administrative problems. The sensitivities of direct and indirect LAMP were 98.2% (166/169, 95% confidence interval [CI] 94.9–99.4) and 98.9% (178/180, 95% CI

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* According to the classification of Miller and Jones: M1 (pure mucus), M2 (little purulent content), P1 (purulent sputum less than one third of the volume), P2 (purulent sputum between one third and two thirds of the volume) and P3 (purulent sputum more than two thirds of the volume).
96.0–99.7) compared to smear- and culture-positives, respectively. They were respectively 55.6% (30/54, 95%CI 43.4–68.0) and 30.2% (13/43, 95%CI 18.6–45.1) using direct and indirect examination of specimens among smear-negative/culture-positives. Table 1 also shows the sensitivities of Cobas Amplicor MTB and TRC Rapid MTB, which were 99.4% (178/179, 95%CI 96.9–99.9) and 100% (145/145, 95%CI 97.4–100) for smear- and culture-positive specimens, and respectively 69.0% (29/42, 95%CI 54.0–80.9) and 62.2% (23/37, 95%CI 46.1–75.9) for smear-negative, culture-positives. The comparison of two different NAA methods for smear-positive and -negative specimens showed a statistically significant difference between indirect LAMP and other methods for smear-negative specimens (vs. direct LAMP, Cobas Amplicor MTB and TRC Rapid MTB; \( P = 0.013, 0.0003 \) and 0.004, respectively).

Table 2 shows the total sensitivity and specificity of diagnosing a person with TB with two sputum specimens. There was no significant difference in specificity between LAMP and other NAAs. Instances with positive direct LAMP and negative Cobas Amplicor MTB were seen in five patients with TB and two with NTM. Positive direct LAMP and negative TRC Rapid MTB were seen in four TB and one NTM patient. Amplicor MTB-positive but LAMP (direct or indirect) negative results were observed in 19 patients. Two false-positive results of direct LAMP were obtained from the patient with aspergillosis. The results were not considered to be bacteriologically false due to previous history and pulmonary TB sequelae.

### Table 1 Results of LAMP, Cobas Amplicor MTB and TRC Rapid MTB tests and other referral examinations*

<table>
<thead>
<tr>
<th>Test</th>
<th>Direct smear-positive</th>
<th>Direct smear-negative</th>
<th>Sensitivity for smear- and culture-positive % (95%CI)</th>
<th>Sensitivity for smear-negative and culture-positive % (95%CI)</th>
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<tr>
<td></td>
<td>MTC MTC MTC MTC</td>
<td>MTC MTC MTC MTC</td>
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<tr>
<td></td>
<td>culture-positive</td>
<td>culture-negative</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>Direct LAMP ((n = 320))</td>
<td>Positive 166 6 (^\dagger) 30 3 (^\ddagger) 205 98.2 (94.9–99.4) 55.6 (43.4–68.0)</td>
<td>Negative 3 13 24 75 115</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total 169 19 54 78 320</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indirect LAMP ((n = 320))</td>
<td>Positive 178 7 (^\dagger) 13 0 198 98.9 (96.0–99.7) 30.2 (18.6–45.1)</td>
<td>Negative 2 14 30 76 122</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total 180 21 43 76 320</td>
<td></td>
<td></td>
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<tr>
<td>Cobas Amplicor MTB ((n = 318))</td>
<td>Positive 178 8 (^\ddagger) 29 1 (^\dagger) 216 99.4 (96.9–99.9) 69.0 (54.0–80.9)</td>
<td>Negative 1 13 13 75 102</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Total 179 21 42 76 318</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TRC Rapid MTB ((n = 244))</td>
<td>Positive 145 3 (^\dagger) 23 1 (^\dagger) 172 100 (97.4–100) 62.2 (46.1–75.9)</td>
<td>Negative 0 10 14 48 72</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Total 145 13 37 49 244</td>
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</tbody>
</table>

* MTC culture: MOTT is categorised as culture-negative as for M. tuberculosis complex isolation.
\(^\dagger\) No growth of MOTT.
\(^\ddagger\) Culture-negative \((n = 7)\), M. kansasii positive \((n = 1)\).
LAMP = loop-mediated isothermal amplification; MTC = Mycobacterium tuberculosis complex; CI = confidence interval; MOTT = Mycobacterium spp. other than M. tuberculosis.

### Table 2 Performance of LAMP, Cobas Amplicor MTB and TRC Rapid MTB tests for the diagnosis of tuberculosis patients

<table>
<thead>
<tr>
<th>Test</th>
<th>Patients diagnosed ((N = 160))</th>
<th>Sensitivity for TB diagnosis % (95%CI)</th>
<th>Specificity for TB diagnosis % (95%CI)</th>
<th>NLR</th>
<th>PLR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TB MOTT Other disease</td>
<td></td>
<td></td>
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<tr>
<td>Direct LAMP</td>
<td>Positive 112 0 2</td>
<td>88.2 (81.4–92.7) 93.9 (80.4–98.3)</td>
<td>0.13 14.55</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Negative 15 18 13</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Indirect LAMP</td>
<td>Positive 106 0 0</td>
<td>83.5 (76.0–88.9) 100</td>
<td>0.17 Infinite</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Negative 21 18 15</td>
<td></td>
<td></td>
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<tr>
<td>Cobas Amplicor MTB</td>
<td>Positive 115 1 1</td>
<td>90.6 (84.2–94.5) 93.9 (80.4–98.3)</td>
<td>0.10 14.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative 12 17 14</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TRC Rapid MTB</td>
<td>Positive 114 0 1</td>
<td>89.8 (83.3–93.9) 97.0 (84.7–99.5)</td>
<td>0.11 29.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative 13 18 14</td>
<td></td>
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</tbody>
</table>

LAMP = loop-mediated isothermal amplification; TB = tuberculosis; MOTT = Mycobacterium spp. other than M. tuberculosis; NLR = negative likelihood ratio; PLR = positive likelihood ratio.
It was speculated that the LAMP test could detect DNA from non-active MTC. Cobas Amplicor MTB was false-positive for two specimens from a patient with pulmonary pneumonia and M. kansasii infection. TRC Rapid MTB was positive for one specimen collected from a patient with bronchiectasis. These could be due to laboratory or bedside cross-contamination. The sensitivity and specificity of the direct LAMP method was comparable with other NAA methods, while the sensitivity of indirect LAMP was slightly lower. Comparisons of the positive and negative likelihood ratios were also similar.

The relationship between the degree of smear positivity of sputum specimens and their NAA test results in terms of positive rates is shown in Table 3 according to the methods used. For simplicity, a linear regression analysis with the positive rate as a subordinate variable and the degree of positivity as an independent variable (as negative = 1, scanty = 2, 1+ = 3, 2+ = 4, 3+ = 5) was performed. According to the $\chi^2$ test for trend, the slope and linearity for all four methods were significant.

DISCUSSION

Due to the HIV epidemic and the emergence of drug-resistant TB, a laboratory test with a short turnaround time (TAT) and high sensitivity is urgently needed. In general, liquid culture shows the highest sensitivity, but it is not easy to set up a culture laboratory for this purpose, mainly due to cost and bio-safety issues. The simple NAA method is therefore the most promising for application even in peripheral laboratories, particularly in developing countries. LAMP is an NAA method that can be performed in one tube under isothermal conditions, and has high amplification efficiency. It has already been demonstrated that the in-house LAMP method has a sensitivity similar to that of culture for the detection of MTC from clinical specimens.

The sensitivity of direct LAMP (88.2%) was somewhat lower than Cobas Amplicor MTB and TRC Rapid MTB; however, the difference was not statistically significant. The sensitivities of direct and indirect smear microscopy for diagnosing TB in this study were respectively 69.3% (176/234) and 74.0% (188/254). The sensitivity of the direct LAMP method was higher than direct smear microscopy ($P = 0.006$), but that of indirect LAMP did not exceed indirect smear microscopy. With a positivity rate in smear-positive cases of nearly 100%, and that in smear-negative, culture-positive cases well over 50%, the LAMP test is considered to have a sensitivity intermediate between the smear and culture examination methods, and its performance is roughly similar to that of other tested NAAs, except for indirect LAMP for smear-negative specimens.

There are several other NAAs already in practical use, such as BD ProbeTec ET (BD, USA), GenoType Mycobacteria Direct (HAIN Lifescience, Nehren, Germany) and Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA). The sensitivity of BD ProbeTec ET for the detection of MTC has been reported as respectively 96.7–100% and 12.5–66.0% for smear-positive and -negative specimens. The sensitivity of GenoType Mycobacteria Direct for the detection of MTC has also been reported as 97.9–98.7% and 80.0–89.6% for smear-positive and -negative specimens. Boehme et al. recently reported a sensitivity of the Xpert MTB/RIF (Cepheid) test of 72.5% for smear-negative, culture-positive specimens. The sensitivity of the direct LAMP test was in line with other NAAs for smear-positive specimens, but was lower for smear-negative specimens. Although direct comparisons still need to be carried out, the present results suggest limitations of the new LAMP test kit and the importance of culture examination for optimal detection of MTC.

The present study indicated significantly lower sensitivity of the indirect LAMP test for smear-negative specimens compared with other NAAs. One possible reason for the low sensitivity of the commercial version of the LAMP test is the small volume of specimen used in the reaction. The Cobas Amplicor MTB uses 100 μl of treated sputum, and approximately 25% of the extracted nucleic acids (v/v) are employed for the amplification. Similarly, TRC Rapid MTB starts from 200 μl, and uses approximately 10%. The LAMP method starts with only 40 μl of sputum and the final volume used for amplification is 1.2 μl, which corresponds to approximately 3% of the original specimen as nucleic acids. The relatively lower positivity rate of LAMP compared with other NAAs
could be due to the smaller amount of target nucleic acids used in the reaction.

The addition of NALC to the specimen was the only difference in the procedure for indirect LAMP compared to direct LAMP. We compared the effect of NALC on LAMP using several sputum specimens, and concluded that this treatment had no effect on the outcome of the test (data not shown). It is conceivable that the centrifugation and homogenisation steps in the indirect test could reduce the concentration of AFB in the specimens, causing differences in the results between the direct and indirect methods. Another possibility is that the laboratory technologists carefully selected the purulent part of the sputum specimens for the test, thus achieving the higher positivity, especially in paucibacillary specimens. Amplification inhibition is a common cause of false-negatives. However, as discrepant results between NAAs occurred mainly with smear-negative samples (Table 3), we concluded that there was little possibility of inhibition.

The population in this study had special features, with a high percentage of active TB cases (79.4%) and some with pulmonary diseases other than active TB, including MOTTs. We documented LAMP performance separately for specimens graded for bacillary load in terms of smear positivity. The performance of LAMP tests remains similar to that of other NAAs, in terms both of sensitivity in smear-negative, culture-positive specimens, and of similarity in the dose-response relationship. It was thus considered that LAMP and other NAAs were fairly evaluated in this study.

As a practical problem, cross-contamination and complicated procedures are major obstacles to the routine use of NAA. The prototype LAMP kit requires nucleic acid extraction, centrifugation, two washes with a buffer, amplification and detection. The new LAMP kit requires neither centrifugation nor target purification by washing with buffer. A further advantage of the new kit is the reduced number of steps, which results in a lower risk of cross-contamination. The amplified product is not removed from the reaction tube, and is disposed of directly without opening the tube. Another advantage is the short hands-on time. The average time required for one test is approximately 1 h, and one additional specimen adds only a few minutes for mixing with extraction reagent and dropping the extracted DNA into the tube. Thus, for example, the time for testing six specimens, including negative and positive controls, will be only 1.5 h.

CONCLUSION
A new commercial version of the LAMP kit was evaluated using clinical specimens for the diagnosis of TB. The new LAMP kit is very simple, rapid and easy to use. The overall performance of the LAMP test was intermediate between the smear and culture methods. This implies that the LAMP test will detect approximately half of smear-negative, culture-positive TB cases, which seems slightly less than other NAAs. The specificity of diagnosing TB among those suspected of having the disease will be essentially 100%, provided laboratory quality is maintained. It will be useful as a primary testing tool for TB, and may provide additional diagnostic value (sensitivity and species specificity) to complement smear examination by microscopy in many health care settings.

Acknowledgement
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References


OBJECTIF : Elaborer pour une utilisation commerciale et évaluer l'efficience en matière de diagnostic de la tuberculose (TB) un nouveau kit de test d'amplification isothermique médié par une boucle (LAMP) comportant un système simple d'extraction de la DNA.

SCHEMA : Le test LAMP a été réalisé en utilisant des échantillons de crachats non traités ou traités par N-acétyl-L-cystéine (NALC) NaOH. On a comparé l'efficience du test avec celle d'autres examens conventionnels de laboratoire, notamment avec d'autres tests d'amplification des acides nucléiques (NAA).

RÉSULTATS : La sensibilité du LAMP dans le cas d'examen de crachats bruts (LAMP direct) dans les échantillons à frottis et à culture positifs a été de 98,2% (IC95% 94,9–99,4) et sa sensibilité dans les échantillons à frottis négatif mais à culture positive a été de 55,6% (IC95% 43,4–68,0). Les sensibilités en matière de diagnostic du LAMP direct pour le diagnostic d'un patient atteint d'une maladie TB a été de 88,2% (IC95% 81,4–92,7). Ces valeurs de sensibilité du LAMP direct ont été légèrement mais non significativement plus faibles que celles du Cobas Amplicor MTB et TRC Rapid MTB, alors que la sensibilité du test LAMP portant sur des échantillons traités par NALC-NaOH a été significativement plus faible que celle d'autres tests NAA (P < 0,05) pour les échantillons à frottis négatif mais à culture positive. La nouvelle version commerciale du kit LAMP est d'utilisation facile et fournit des résultats dans l'heure qui suit la réception des échantillons de crachats.

CONCLUSIONS : Ce test est considéré comme un outil prometteur pour le diagnostic de la TB, même dans les laboratoires périphériques à équipement limité comme ceux des pays en voie de développement.

RÉSUMÉ

OBJETIVO: Examinar el rendimiento de un nuevo estuche diagnóstico de amplificación isótérmica de asas de ADN (LAMP) distribuido comercialmente, que comporta un dispositivo sencillo de extracción del ADN destinado a detectar la presencia del complejo Mycobacterium tuberculosis.

MÉTODO: Se aplicó el LAMP en muestras de esputo tratadas con N-acetil-L-cisteína e hidróxido de sodio (NALC-NaOH) y en muestras sin tratamiento previo. Se comparó la eficacia máxima del estuche con la eficacia de otras pruebas de laboratorio convencionales, entre ellas otras pruebas de amplificación de ácidos nucleicos.

RESULTADOS: La sensibilidad de la prueba LAMP a partir de esputo sin tratamiento (LAMP directo) en muestras con baciloscopia y cultivo positivo fue 98,2% (IC95% 94,9–99,4) y la sensibilidad en muestras con baciloscopia negativa y cultivo positivo fue 55,6% (IC95% 43,4–68,0). La sensibilidad diagnóstica global de la prueba LAMP directa en personas con enfermedad tuberculosa fue 88,2% (IC95% 8,4–92,7). Esta sensibilidad es ligeramente menor a la sensibilidad de las pruebas Cobas Amplicor MTB y TRC Rapid MTB, pero la diferencia no presenta significación estadística. Al contrario, la prueba LAMP indirecta practicada en muestras de esputo tratadas con NALC-NaOH ofreció una sensibilidad significativamente inferior a las demás pruebas de amplificación de ácidos nucleicos (P < 0,05) en muestras con baciloscopia negativa y cultivo positivo. La nueva versión comercial del estuche diagnóstico LAMP fue de manipulación sencilla y se obtuvieron los resultados en la primera hora después de la recepción de las muestras.

CONCLUSIÓN: Se considera que la prueba LAMP representa un instrumento promisorio en el diagnóstico de la tuberculosis, incluso en los laboratorios periféricos con equipos escasos como existen en los países en vía de desarrollo.