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Review

Combining LAMP and Au-Nanoprobe to detect INH-RIF resistance accurately in tuberculosis: an evidence-based review

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Abstract

Approximately 1.41 million people die annually due to tuberculosis. One of the main problems in Tuberculosis eradication is the development of resistance to various antibiotics. However, current efforts to detect resistances face challenges such as limited equipment, budget, and time. This evidence-based review investigated loop-mediated isothermal amplification, an alternative molecular diagnostic tool with promising performance and applicability in developing countries, and its use combined with Au-Nanoprobe to detect antibiotic resistance in tuberculosis. The literature search was conducted through four databases (Proquest, EBSCOhost, Scopus, and Pubmed) for useful articles on loop-mediated isothermal amplification and Au-Nanoprobe in detecting tuberculosis and tuberculosis resistance. After filtering the result with inclusion and exclusion criteria, the search produced three papers that best answer the clinical question. Loop-mediated isothermal amplification amplifies a target sequence, and Au-Nanoprobe responds to the DNA specific to the target mutant, producing an observable color change. Loop-mediated isothermal amplification and Au-Nanoprobe showed 100% sensitivity and specificity in detecting rifampicin and isoniazid resistance. Another study investigated its viability to detect tuberculosis and found 98.2% sensitivity and 88.2% specificity. Combining loop-mediated isothermal amplification and Au-Nanoprobe had a shorter time to get results and should also be relatively cheaper because it does not need a high temperature to work and requires less equipment. In conclusion, loop-mediated isothermal amplification and Au-Nanoprobe can be used as an efficient and accurate method to detect isoniazid and rifampicin-resistant tuberculosis strains. The new technology is promising for developing countries due to their high disease burden but facing several healthcare barriers.

Key words: Multidrug-resistant tuberculosis; diagnosis; loop-mediated isothermal amplification; Au-Nanoprobes; isoniazid; rifampicin.

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Introduction

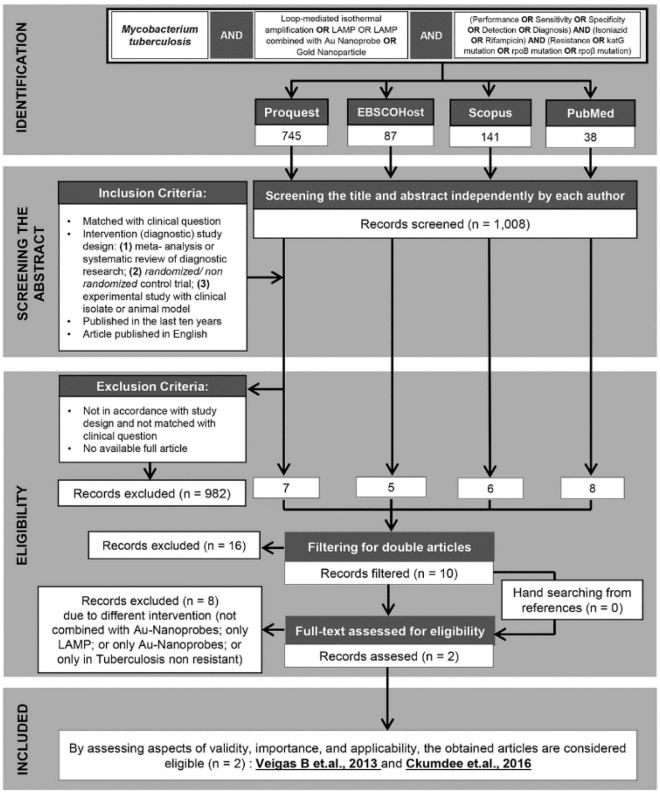
Tuberculosis (TB) is a burden on a global scale. There were 10 million TB cases and 1.408 million deaths in 2019 worldwide, including 208,000 TB deaths among positive Human Immunodeficiency Virus (HIV) cases [1]. In 2019, TB was most prevalent in Southeast Asia (44%); the top five countries contributing to TB in the world are from Asia, including India (26%), Indonesia (8.5%), China (8.4%), Philippines (6.0%), and Pakistan (5.7%). In 2019, by an estimated epidemiological burden, Indonesia, our home country, was in the second-highest for the number of TB incidence, eighth place of TB with HIV, and fifth place for estimated incidence of multidrug-resistant (MDR) or rifampicin-resistant tuberculosis (RR) TB [1]. In the same year, global estimates suggest 3.3% of new cases and 18% of previously treated cases had MDR/RR-TB, with 361,000 patients with RR and isoniazid (INH)-resistant; 105,000 patients that are RR only; and 1.06 million patients with INH monoresistant [1]. World Health Organization (WHO) conducted several political strategies to eradicate the TB epidemic, such as mobilizing universal access to TB diagnosis. There is still a large gap between the number of new cases reported and the estimated incident cases due to underreporting of detected cases and underdiagnosis [1].

WHO has established culture-based phenotypic drug susceptibility testing (DST) as a gold standard for diagnosing TB resistance [2]. However, bacteria culture requires an expensive contamination safety facility, repeated visits to the clinic, and increases the patients' burden through transport expenditure [3]. The long time it takes for the culture to grow also causes many patients to not return for follow-up [3]. Hence why the current approach recommended by WHO to detect resistant MDR/RR-TB is via rapid molecular test or sequencing [1]. It is known that Xpert MTB/RIF, with a sensitivity of 85% (82-88%) and a specificity of 98% (97-98%), can determine resistance status against rifampicin, but

not isoniazid [4]. Other option includes Line Probe Assay (LPA) and its variants, such as Hain GenoType MTBDRplus (v.1.0 and 2.0) and Genoscholar NTM + MDRTB [5]. The former fare better than Xpert as it detects isoniazid resistance; it also has better sensitivity and specificity for rifampicin resistance, with 98.2% and 97.8%, respectively [6]. In contrast, for isoniazid, it was 95.4% and 98.8% [6]. The latter begets even better results with sensitivity and specificity of 96.5% and 97.5%, respectively, for rifampicin as well as 94.6% and 97.6% for isoniazid [7]. LPA is known to be labor-intensive and requires more time to result than GeneXpert [8]. Although the provision of equipment and operators in molecular tests still serves as an obstacle for its usage, it currently sits as the go-to method to detect resistances [5,7,9,10]. The cost is also an ever-present thorn on diagnosis and treatment, accumulating to 20 billion USD per year and still leaving thirty-three percent of the 10 million TB patients undiagnosed [5].

Diagnostic delay and resource limitation will significantly impact treatment initiation and increase the disease's transmission period. Today, nanotechnology development has grown to a point where it is applicable in healthcare settings. Au-Nanoprobe (Au-Np) has a unique optic figure, allowing it to change color when interacting with ssDNA. Using Au-Np gives the advantage of cost-efficiency and ease of application; mere visual observation is used instead of conventional fluorescent and radioactivity-based assay. Before the utilization of Au-Np, the sample DNA must first be amplified. The amplification method can be improved by utilizing Loop-mediated isothermal amplification (LAMP) instead of conventional polymerase chain reaction (PCR). LAMP is shown to be more specific, and sensitive, as well as more cost-effective and time-efficient than PCR. This review aims to examine the combination of amplification by LAMP and detection by Au-Np as a promising diagnostic tool for TB and TB resistance in the future [11–14].

Figure 1. A flowchart of the selected article process. Figure design by the authors.



Methodology

This evidence-based review searched for the latest sources relating to rapid, low-cost, and efficient diagnostic resistant TB strategy, LAMP. The development of LAMP in diagnosing TB produces novel approaches by combining gold nanoparticles for visualizing the result of detection and increasing rapidness, sensitivity, specificity in detecting resistant TB. We use the clinical question in the "PIO" formula to conduct a searching strategy, Population: Resistance TB; Intervention: LAMP-AuNP; and Outcome: Diagnosis of INH-RIF resistance. Literature searching through Proquest, EBSCOHost, Scopus, and Pubmed with the keyword "*Mycobacterium tuberculosis* AND (Loop-mediated isothermal amplification OR LAMP OR LAMP combined with Au Nanoprobe OR Gold Nanoparticle), AND (Performance OR Sensitivity OR Specificity OR Detection OR Diagnosis) AND (Isoniazid OR Rifampicin) AND (Resistance OR *katG* mutation OR *rpoB* mutation OR *rpoβ* mutation)" was performed with inclusion criteria: articles in level evidence 1a-4, written in English, and published within the last ten years. Studies that are only available in abstract or do not answer the clinical questions were excluded. The search was done in December 2020, and we obtained 1,008 hints from four journal databases. We selected three experimental studies that best answer the clinical question; a study from Thailand which explains the development of LAMP with the addition of Au-NP as a novel strategy in diagnosing TB, and two studies (from Portugal and Thailand) that use LAMP with Au-Np to detect rifampicin and isoniazid resistance [12,13,15]. A flowchart of the selected article process can be seen in Figure 1.

Furthermore, we searched supporting literature published about LAMP's effectiveness with/without Au-Np, Au-Np alone, and LAMP alone compared to

other diagnostic tools. We obtained 25 eligible articles from 200 hits and assessed the level of evidence to be 1-2 in reference to the Oxford Centre for Evidence-Based Medicine (CEBM) guideline. Additional 38 studies of different diagnostic tools are also included for comparison. For this purpose, we do not exclude literature published more than ten years prior due to the limited available data. After everything has been found, synthesis starts from the primary literature to its supporting literature.

Results and discussion

Drug-resistant tuberculosis

Multidrug-Resistant Tuberculosis (MDR-TB) is an instance of *Mycobacterium tuberculosis* showing resistance to both isoniazid and rifampicin, regardless of its resistance status to other anti-TB antibiotics. When facing a case of MDR-TB, we are to consider second-line anti-TB medications, which are generally less preferable due to their lesser effectiveness compared to the first-line drugs and their side effects [16]. Moreover, there is also Extensively Resistant Tuberculosis (XDR-TB), which resists isoniazid, rifampicin, and fluoroquinolone, one of the second-line anti-TB drugs [16].

Isoniazid monoresistance is the most common mutation for the *M. tuberculosis* strain. In vivo, isoniazid is activated by peroxidase and catalase enzyme coded by the *katG* gene [16,17]. Mutation of this gene occurs in 50% of the isoniazid-resistant strain. Among those mutants, most mutations are of Ser315Thr, which causes a reduction in the enzyme's activity. It is known that Ser315Thr mutants have reduced catalase activity up to six-folds, and peroxide activity reduced to less than half of the wild types. In a milder isoniazid resistance, the mutation is found in promotor *inhA*, *acpM*, and *kasA*. These mutations are thought to cause protein overexpression, rendering isoniazid ineffective [16,17].

Rifampicin-resistant isolates have mutations that change the sequence of RNA polymerase's beta subunit 27-amino acid region. A study found that more than 95% of the rifampicin-resistant strain have specific mutations on *the rpoβ* gene in region 81bp. Changes of codon526 or codon531 most commonly cause high levels of resistance. Another cause is an amino acid substitution; the most common are Ser531Leu (42%) and His526Tyr (23%) [16]. Mutations on codons 511, 516, 518, and 522 have been shown to cause low rifampicin and rifapentine and sensitivity to rifalazin and rifabutin [12,16].

Selection of the proper diagnostic tool

The increasing number of rifampin and isoniazid resistance globally has led researchers to compete in developing the most effective and efficient molecular diagnostic techniques for detecting RR, INH-monoresistance, MDR, or XDR patients [1,5]. It is known that DNA sequencing has been regarded as the gold standard for molecular diagnosis, as it is proven to be superior to molecular methods [5,12,16]. Several aspects are considered when proposing a new diagnostic tool. The first is accuracy and effectiveness, which are shown in existing studies. Next, the time taken until the interpretation of results and cost for maintenance or usage for the population is accounted as essential determinants of the tools' efficiency. Other variables brought to the account are applicability, regulation, quality control, and the required technicians' capacity to use the device [5]. One known diagnostic method that may fulfill these criteria is LAMP, which has recently been shown to detect resistances [18]. Its applicability in developing countries, short duration of testing, and estimated low cost make for a great candidate for the future of diagnostic tools for TB resistance in rural areas [12,13].

Loop-mediated isothermal amplification and The Working Principle

Notomi *et al*. [14] first introduced a method to amplify DNA in an isothermic process called Loop-Mediated Isothermal Amplification (LAMP) in 2000. The method boasts simplicity and efficiency compared to PCR by requiring only a constant temperature of around 60-65 °C and has a comparable diagnostic accuracy, if not superior, to smear microscopy [12,15,81]. This method's high specificity is owed to four primers' DNA recognition at the start of the amplification and two at the next stage [14].

The initial stage uses outer and inner primers to create complementary strands to the target DNA. The outer primer is shorter and will release a complementary strand that will form a loop structure. The structure allows DNA synthesis from the backward inner primer. The produced DNA will be a stem-loop to which cycling can begin. The LAMP cycling starts with hybridization of the forward inner primer to generate a one-gapped stem-loop DNA. The following DNA formed will create another complementary structure of the stem-loop DNA and additional double copies of the target sequence. The cycles continue and will generate numerous stem-loop DNAs and cauliflower-like structures with multiple loops [14,19]. Thus, as the process continues over time, more initiation sites

emerge, which increases target detection. The amplification product can be mixed with binding dyes, such as SYBR green, to allow detection using fluorescence detectors; this results in fluorescent signals being detectable, either by naked eye with UV light or by using spectrophotometers [14,20].

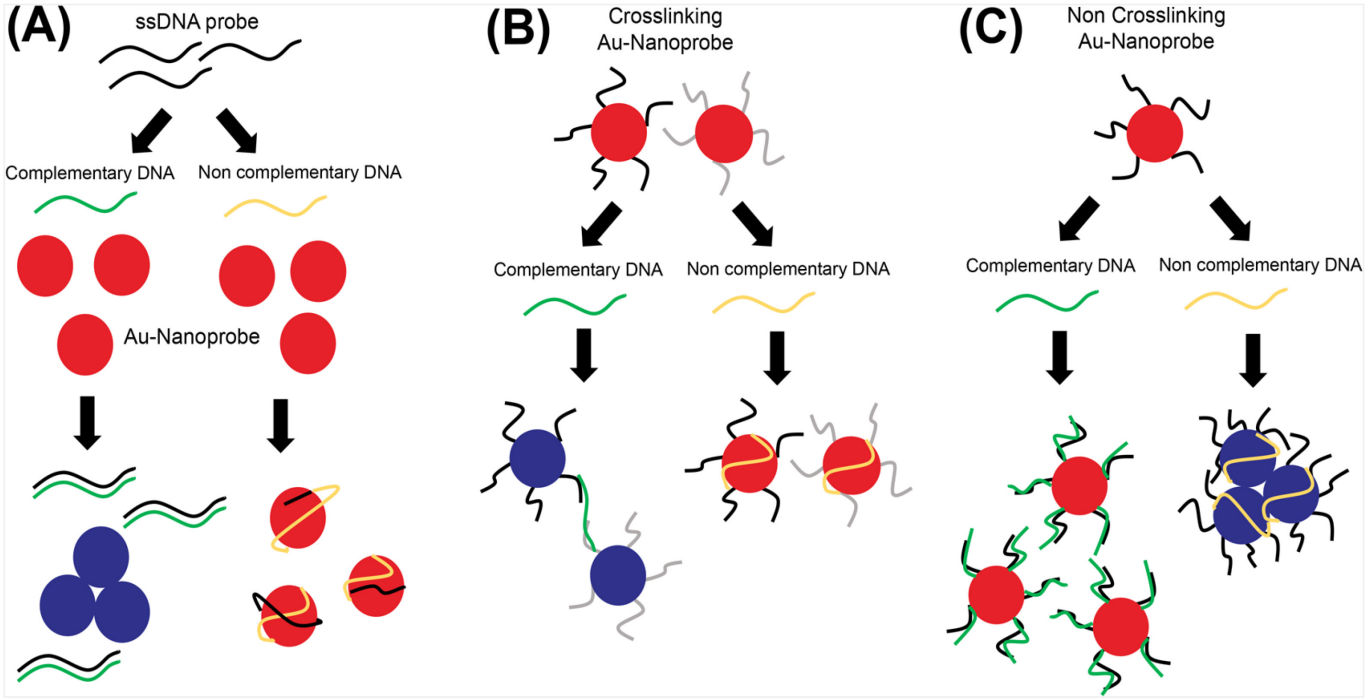
In TB diagnosis, LAMP has been acknowledged as a valuable tool for TB detection but not for resistant TB identification [7]. However, a study by Takarada *et al*. [18] shows the possibility for that to change. The study remarks that LAMP can be used with DNA chromatography to detect up to four mutations, which manifests as rifampicin resistance. The LAMP reaction is carried out with primers corresponding with the wild-type and mutants, totaling eight primers if the goal is to seek out all four possible mutations. Next, the amplification product is diluted with distilled water 100-fold and then mixed with 2 µL of streptavidin and 10 µL of developing solution. Detection is then done by dipping the Chromatography Printed-Array Strip (C-PAS) membrane strip for 15-20 minutes, after which blue lines will appear in the presence of mutations [18].

LAMP and Au-Np combination for detection rifampicin and isoniazid resistance

When combined with Au-Np, LAMP works conventionally, amplifying a target sequence. Next, detection would be optimized by applying Au-Np. Although Au-Np has yet to be regulated through guidance policy, several studies have been made to see Au-Np performance as a diagnostic tool. In 2009, Soo *et al*. [21] used Au-Np to improve MTB detection after amplification via PCR. Au-Np used were tied to primer IS6110 and Rv3618 and compared to culture as reference. Au-Np IS6110 has a 96.6% sensitivity and specificity of 98.9%, while Rv3618 has 94.7% and 99.6%, respectively [21]. Since then, new Au-Np methods have been developed, such as the paper-based assay with Au-Np colorimetry developed by Tsai *et al*. [22].

Au-Np aids in detection with its ability to change color depending on their distance from each other. The surface plasmon resonance (SPR) of Au-NP causes a redshift when further apart from each other, tugging the color into the red spectrum (longer wavelength). [13,23] Hence, on dispersion, Au-Np gives off red color (long wavelength), while on aggregation, Au-Np gives off violet (short wavelength). Colorimetric interpretation of this reaction can then be used to determine if Au-NP disperse or aggregate in response to a sample. Result interpretation depends on whether

Figure 2. A different method of DNA detection using Au-NP. (A) unmodified Au-NP, The Au-NP is left unattached to any ssDNA probe and aggregates when complementary DNA binds to the ssDNA probe instead of the Au-NP. (B) Crosslinking modified Au-NP, two batches of Au-NP each with one half of the ssDNA probe is used, and complementary DNA will bind on the two halves, which induce aggregation. (C) Non-Crosslinking Au-Nanoprobe, ssDNA probe is attached to Au-NP, complementary DNA will prevent aggregation [23,27,28]. Figure design by the authors



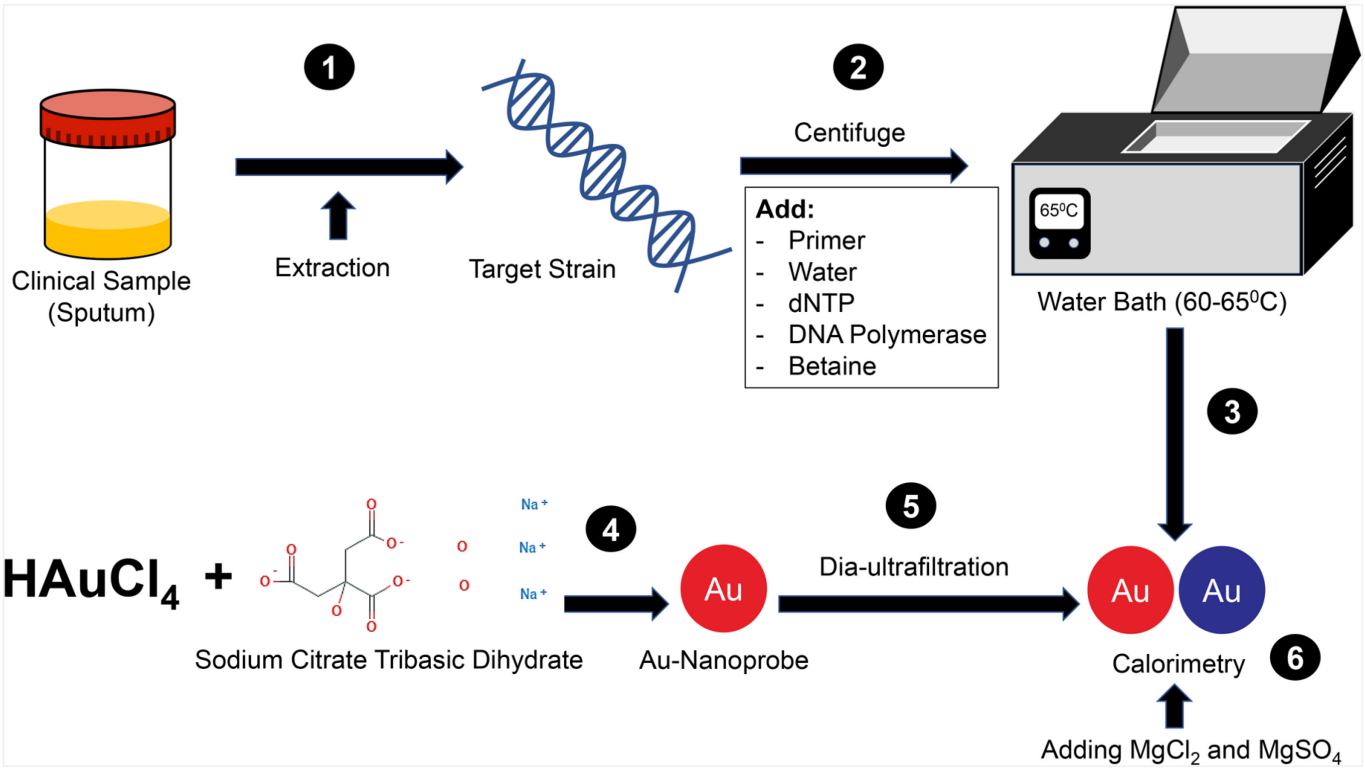
or not a modification is done to Au-Np as described in Figure 2. [12,21,22].

In application, Au-Np can be utilized via modifications or by mixing it with specific DNA probes. The mechanism behind the two is as follows:

1. Au-Np can be used without modification by mixing it with a single strain DNA probe. When there is complementary DNA, the single strain DNA will bind together, causing Au-Np to not bind with sample DNA or the single strain DNA probe. Au-Np will then aggregate when salt is added. When a non-complement DNA is present, the single strain DNA probe and the sample DNA will not bind together, but instead, Au-Np will absorb the single strain DNA probe, which prevents aggregation [27].
2. Au-Np can also be modified to react with specific complementary DNA. This modification can be done in two ways:
   a. Using two separate batches of modified Au-Np, each with half of the complementary DNA sequence. When there is a sample DNA carrying sequence matching the line carried by the two Au-Np, hybridization between the complement DNA and two nanoprobes will occur, which leads to aggregation. If the sample DNA is non-complementary, no hybridization occurs, retaining the Au-Np in a dispersed state. This method is referred to as crosslinking Au-Np [28].
   b. Using one batch of Au-Np modified to carry a specific complement DNA (targeted sequence), causing the target DNA from the sample to not aggregate. However, if there is no target DNA in the sample, the modified Au-Np will aggregate. This method is referred to as non-crosslinking Au-Np [23].

The Non-crosslinking Au-Np technique is known to have a quicker aggregation reaction than crosslinking Au-Np (3 minutes instead of 10 minutes). The former is also relatively practical, as it only requires one batch of Au-Np while retaining the same detection capability. However, do note that Au-Np requires a lot of aggregated target DNA to be effective; approximately

Figure 3. Proposed steps for detection using LAMP and Au-Np combination. (1) Target strain is extracted from sputum sample; (2) Target strain is put on centrifuge with all necessary addition for LAMP reaction (the outer and inner primers, dNTP, DNA polymerase, betaine) the sample is then put on a water bath for LAMP for 30-60 minutes at around 60-65 oC; (3) Sample is ready for detection via Au-Np; (4) Au-Np is synthesized by mixing HAuCl4 with distilled water and subsequently add Sodium citrate tribasic dihydrate; (5) Synthesized Au-Np can be conjugated with thiolated DNA probes, and then undergo dia-ultrafiltration before applied; (6) MgCl2 or MgSO4 salt is added to induce aggregation in the absence of a complementary DNA, colorimetry is used to interpret result [12–14,20,30]. Figure design by the authors.



200 DNA molecules are needed for each aggregated nanoparticle, hence why DNA amplification was necessary [23]. For application purposes, it may be worth noting that Au-Np could also increase discrimination to mismatch on 3' ends. To maximize discrimination, Au-Np should have a density of 24 pmol/cm^2 at room temperature [24]. For determining TB resistance, non-crosslinking Au-Np with dia-ultrafiltration purification is the most promising, specifically for isoniazid and rifampicin resistance detection.

The most critical step involves the LAMP amplification of a *katG* gene,[12] or *rpoB* gene fragment,[13] followed by its hybridization using specific Au-nanoprobes, which will be explained below.

DNA extraction and sample amplification with LAMP

Sample DNA is first extracted from the specimen by suspending it in 300 µL distilled and then incubated at 95 °C Thermoblock for 20 minutes, plus an additional 15 minutes treatment using an ultrasonic bath. The sample will then be treated on a centrifuge for 5 minutes at 10,000 Xg before it is ready for amplification using LAMP. The amplification requires an outer primer, inner primer, DNA polymerase, dNTP, betaine, MgSO4, and plasmid template. The optimum temperature for the LAMP reaction is 61°C; this temperature is maintained for 60 minutes. After which, the LAMP reaction is halted by heating up to 95 °C for 2 minutes [12].

Gold Nanoprobe (Au-Np) synthesis

The nanoparticle formation begins by mixing hydrogen tetrachloroaurate trihydrate with water while boiling before adding sodium citrate tribasic dihydrate. Then the resulting mixture should be cooling off to room temperature. Color change consistent with the aggregation should then be observable [23]. Nanoprobe can then be purified by dia-ultrafiltration using cellulose membrane with ten kDa molecular weight cut-off. A pressurized dead-end permeation system should run through the membrane so that transmembrane pressure reaches 0.1 bar. This process results in nanoprobes that are more specific to *M. tuberculosis* and is more efficient [24].

Au-Np targets the *katG* mutant gene on the isoniazid-resistant strain and codon 511-531 mutation on the *rpoβ* gene on the rifampicin-resistant strains [12,13]. Amplified DNA will be incubated with Au-Np and then purified [29]. Two batches of Au-Np will test the sample: one to detect wild type (WT) and the other to detect resistance genes, as shown in Figure 3. The stability of the bond between probe and WT gene can then be evaluated using colorimetry [12,13,21].

As explained, aggregation of Au-NP results in a redshift which can be quantified by measuring the absorbance. Tsai *et al*.[22] reported that the shift from the default 520 nm to more or equal to 600 nm. Thus, after amplified with LAMP, and subsequent treatment with Au-NP and the salt to induce possible aggregation, it is possible to determine the absorbance of the prepared sample with colorimetry [12,21].

Effectivity of LAMP and Au-Np in the detection of isoniazid and rifampicin resistance

A study by Kaewphinit *et al*. [15] compares LAMP and Au-Np with culture as a gold standard and acid-fast bacilli (AFB) smear test as a reference to evaluate the ability to detect *M. tuberculosis* infection. They found that the sensitivity was 98.2% in raw sputum samples but only 55.6% in culture-positive/smear-negative samples, while specificity was found to be 88.2% [15]. The study, however, did not evaluate its ability to detect antibiotic resistance. We have yet to find studies regarding LAMP and Au-Np combination for resistance diagnosis that use culture or smear as a reference. However, existing studies show promising potential. Research by Pedrosa *et al*. [10] found that this method can detect resistance to rifampicin and isoniazid in 25 samples with 100% sensitivity and specificity compared to commercially available screening kits. Veigas *et al*. [13] also found the same result (100% sensitivity and specificity), although with a smaller sample size of twelve. More recently, a study by Ckumdee *et al.* [12] uses a sample of 46 and found that the method also has 100% sensitivity and specificity compared to Genotype MDR-TB Plus. However, LAMP itself has significantly lower specificity on smear-negative samples, 40.3% (95% CI = 27.9–54.0) to 42.2% (95% CI = 27.9– 57.9, which may affect how effective LAMP and Au-Np [12,13,20]. Though it appears low, this number is not too far off from the currently available LPA, which reduced its interpretability to 44% when faced with the smear-negative specimen instead of 95% in smear-positive samples [31].

The use of LAMP also boasts overall higher efficiency than existing methods is represented in Table 1. On average, standard commercial LAMP may have the same efficiency as other molecular diagnostic methods but is still more economical. According to a study by Liu *et al*.[11] compared to real commercial time PCR, LAMP by itself also costs relatively less

while maintaining the same amount of analysis time; however, it can only process 16-96 tests per run instead of PCR's 16-384. The article also describes the use of a capillary-array microsystem, which trades the number of tests per run (ten) for shorter analysis time (approximately 50 minutes as opposed to 120) and portability [11]. WHO policy recommendations state that LAMP may thus be used to replace sputum smear microscopy and follow-up tests to smear microscopy for adults [20]. However, they also remarked that LAMP should not yet be used to detect rifampicin resistance in populations with MDR-TB risk, although the recommendation itself has only acknowledged LAMP as a method to detect the presence of TB rather than whether or not resistance is present [20].

As for accuracy, compared to other molecular diagnostic methods, the capacity of LAMP and Au-Np to detect resistances is less comparable due to the lack of data comparing LAMP and Au-Np with the gold standard. Regardless, current data shows LAMP and Au-Np can potentially rival LPA, the specificity and sensitivity of which are generally higher than other methods bar LAMP and Au-Np, even reaching excellent specificity for rifampicin [61]. However, LPA is also known to be relatively inefficient. It is a labor-intensive procedure requiring aids from a regional or reference laboratory. Transport of sample to laboratory also causes an increase in time-to-result and the chance of administrative errors [62].

Table 1. A summary of the comparison of monetary cost and time-to-result between tuberculosis resistance diagnostic methods.

Method	Sample	Estimated time	Sensitivity (%); CI 95%		Specificity (%); CI 95%		Estimated Cost
			Isoniazid	Rifampicin	Isoniazid	Rifampicin	
Culture-based phenotypic DST [2,3]	Clinical isolate [2,3]	3-4 weeks [2]	Gold Standard [2,3]				1.63-62.01 USD [3]
Standard Commercial LAMP [20]	Clinical respiratory isolate [20]	120 mins [20]	Not yet data available				13.78–16.22 USD [20]
LAMP Au-Np [12,13]	Clinical respiratory isolate [12,13]	Estimated 75 mins [12]	100 [12]*	100 [13]*	100 [12]*	100 [13]*	Not data, but stated as low cost [12,13]
MODS [32,33]	Sputum [32,33]	14,3 days [32]	97.4 [32]	90.5 [32]	93.8 [32]	87.5 [32]	10.35-43.56 USD [34]
REMA [35–40]	Sputum [35–40]	8-9 days [35–40]	96% (94-98) [35]	97% (95-98) [35]	96% (95-98) [35]	99 (98-99) [35]	± 3 USD [36]
Real Time PCR [11,41]	Sputum [11,41]	120 mins [11]	66.6 [41]	62.5 [41]	95 [41]	80 [41]	± 26 USD [42]
Multiplex PCR (MID-DRS) [43–46]	Sputum [43–46]	2-3.5 hours [43, 46]	76.5 [43]; 82.3 [44]	97.2 [43]; 97.9 [44]	100 [43, 44]	96 [43]; 100 [44]	Estimated 6 USD [45]**
Xpert MTB/RIF [20,47,48]	Sputum [20,47,48]	108-120 mins (nonultra) [47,49] 65-77 mins (ultra) [49,50]	-	85 (82-88) [4]	-	98 (97-98) [4]	10 [7]–28.34 [20] USD
Hain GenoType MTBDRplus v1.0. [6,48]	Sputum [6,48]	6 hours [48]	94.4 (90.2-97.2) [6]	97.1 (93.3-99,0) [6]	96.4 (93.2-98.3) [6]	97.1 (94.3-98.7) [6]	± 10 USD [51]
Hain GenoType MTBDRplus v2.0 [6,48]	Sputum [6,48]	6 hours [48]	95.4% (91.5-97.9) [6]	98.2% (95-99.6) [6]	98.8% (96.5-99.8) [6]	97.8% (95.3-99.2) [6]	± 8 USD [6]
LPA [52]	Sputum [52]	2 days [52]	99.5% [52]	99.5% [52]	98.8% [52]	100% [52]	Approximately less than 50% cost for culture [52]
Genoscholar NTM + MDRTB II [7,53]	Sputum [7,53]	1 day [7,53]	94.9% [7,53]	96.5% [7,53]	97.6% [7,53]	97.5% [7,53]	16 USD [7,53]
Abbott Real Time MTB RIF/INH Resistance [7,54]	Sputum [7,54]	60 [55]-150 [49] mins	88.3 [7,54]	94.8 [7,54]	94.3 [7,54]	100 [7,54]	Not yet available
			88 (82–93) [55]		99 (96–99) [55]		
Xpert MTB/XDR [7,56]	Sputum [7,56]	< 90 mins [50]	83.3 [7,56]	-	99.2 [7,56]	-	Not yet available
BD MAX MDRTB (RIF/INH) [7,57]	Sputum [7,57]	30 mins [57]	82 (63–92) [57]	90 (60–98) [57]	100 (98–100) [57]	95 (91–97) [57]	Not yet available
Cobas MTB-RIF/INH [7,58]	Sputum [7,58]	140 mins [49]	96.9 (93.3-98.8) [58]	97.2 (93-99.2) [58]	99.4 (98.3-99.9) [58]	98.6 (97.2-99.4) [58]	Not yet available
FluoroType MTBDR v2.0 [7,59]	Sputum [7,59]	2.5 hours [60]	91.7 (83.6-96.6) [59]	98.9 (93.8-100) [59]	100 (96-100) [59]	100 (96-100) [59]	Not yet available

Au-Np: Gold Nanoparticle; DST: drug susceptibility testing; INH: Isoniazid; LAMP: loop-mediated isothermal amplification; LPA: Line Probe Assay; MID-DRS: Mycobacterial Identification and Drug Resistance Screen; MODS: microscopic observation drug susceptibility testing; MTB: *Mycobacterium tuberculosis*; MDRTB: Multidrug Resistance Tuberculosis; MTBDR: *Mycobacterium tuberculosis* Drug Resistance; PCR, Polymerase Chain Reaction; REMA: Resazurin microtiter assay; RIF: Rifampicin; *Comparison is stated to be against BACTEC MGIT 960 and INNO-LiPA for rifampicin and GenoType MDR-TB Plus for isoniazid, instead of the gold standard; ** Data come from Multiplex PCR (MID-DRS), which for detection M. tuberculosis only and not stated for resistant strain.

Another method to compare is Xpert MTB-Rif, which beget results at a decent 120 minutes and can detect rifampicin resistance with an excellent 98% specificity and a modest 85% sensitivity [3,9,47,63]. Though LAMP and Au-Np still have the advantage in detecting isoniazid resistance and potentially better accuracy in detecting rifampicin resistance.

As for Au-Np, a study by Ng *et al*. [64] shows that the use of colloidal gold nanoparticles is also relatively inexpensive, costing under 10 USD per run for the entire assay and will do so in under 90 minutes. Hussain *et al*. [65] showed that unmodified gold nanoparticles could obtain results within an hour. More studies should be done to look at the overall applicability of LAMP and Au-Np. However, current findings regarding the two components point that it should be viable for resource-limited settings.

Application of LAMP and Au-Np for developing countries

Currently, there is no available WHO endorsement or policy about LAMP combination with Au-Np for TB resistance. WHO has only published a Policy Guideline for LAMP usage in TB diagnosis, while also remarked that it is yet to be used to detect antibiotic resistance. WHO shows the cumulative result of 20 studies from 2012 to 2016; pooled sensitivity of LAMP was 77.7% to 80.3%, which is already higher than sputum smear

Table 2. Studies of LAMP as a diagnostic method for tuberculosis detection.

No	Study	Country	LoE	Sample	Comparison	Findings
1.	Nguyen VAT *et al*., 2018 [70]	Vietnam	1b	503 sputum samples from patients who visited the TB unit of Ung Hoa district primary health center.	AFB smear and Xpert MTB/RIF; culture as a reference.	LAMP sensitivity and specificity were 45.5% and 95.1% respectively; Xpert MTB/RIF were 87.9% and 99.3%; AFB smear were 45.5% and 98.9%
2.	Nliwasa M *et al*., 2016 [71]	Malawi	1b	773 sputum samples from patients aged 15 and above with chronic cough.	FM microscopy and Xpert MTB/RIF; culture as a reference.	LAMP overall sensitivity and specificity were 65% and 100%, respectively; Xpert MTB/RIF was 77% and 95.3%; FM microscopy was 87.5% and 100%
3.	Phetsuksiri B *et al*., 2020 [72]	Thailand	1b	204 sputum samples from patients with TB symptoms	Xpert MTB/RIF; MTB culture as a reference; also directly compare with Xpert MTB/RIF as reference.	With MTB culture as a reference, LAMP and Xpert's sensitivity was 82.4% and 86.9%, respectively; both have 100% specificity. With Xpert as a reference, LAMP sensitivity was 94.7%, while specificity was 100%.
4.	Habeenzu C *et al*., 2015 [73]	Zambia	1b	200 sputum samples from suspected TB patients.	Xpert; culture and smear as reference.	About smear-positive/culture-positive samples, Sensitivity for LAMP and Xpert was 96.8% and 100% respectively; specificity was 96.5% and 94.5% respectively.
5.	Baikunje N *et al*., 2019 [74]	India	1b	24 fine needle aspirate of a lymph node from HIV-infected patients with tubercular lymphadenitis and 26 from HIV-infected patients with lymphadenopathy from other causes as control.	GeneXpert MTB/RIF and multiplex PCR; culture and smear as reference.	Sensitivity for LAMP, multiplex PCR, and GeneXpert was 79.17%, 91.67%, and 79.17%; Specificity were 100% for LAMP and multiplex PCR and 96.15% for GeneXpert.
6.	Gelaw B *et al*., 2017 [75]	Ethiopia	1b	78 sputum samples from presumptive TB patients.	Culture and smear as reference.	Overall sensitivity and specificity of LAMP were 75% and 98%, respectively.
7.	Yan L *et al*., 2016 [63]	China	2a	A systematic review of 25 articles of LAMP, Xpert MTB/RIF, or SAT as a diagnostic tool for pulmonary TB.	SAT and Xpert MTB/RIF; culture as a reference	Pooled sensitivity and specificity for LAMP were 93% and 94%, 96% and 88% for SAT, 89% and 98% for Xpert.
8.	Yu G *et al*., 2018 [66]	China	2a	A meta-analysis of 14 articles of LAMP as a diagnostic tool for extrapulmonary TB.	CRS and culture as reference	About CRS, Pooled sensitivity of LAMP was 77%, and specificity was 99% with an AUC of 0.96. Regarding culture, it was 93% and 77%, respectively, with an AUC of 0.94.
9.	Deng S *et al*., 2019 [42]	China	2a	A systematic review of 59 articles of LAMP as a diagnostic tool for pulmonary TB in China.	Xpert MTB/RIF, LPA, CPA, SAT-TB, PCR; culture and smear as reference	Pooled sensitivity and specificity of LAMP was 90% and 93% respectively; 87% and 94% for LPA; 90% and 93% for PCR; 79% and 72% for SAT-TB
10.	Nagai K *et al*., 2016 [68]	Japan	2a	A systematic review of 26 studies with sputum samples, one research of extrapulmonary specimen.	Culture as a reference	Pooled sensitivity and specificity for sputum sample studies were 89.6% and 94%, respectively. One extrapulmonary TB study shows a sensitivity of 93% and specificity of 92%.
11.	Pham TH *et al*., 2018 [76]	Peru, South Africa, Brazil, Vietnam	2b	1036 sputum samples from TB-suspected adults.	Xpert mTB/RIF; gold standard: culture and ZN and FM direct smears as reference	The overall specificity of LAMP and Xpert were 98.7% and 97.2%, including follow-up. Sensitivity in culture-positive were 75.6% and 78.5%.

Table 2 ***(continued)*****.** Studies of LAMP as a diagnostic method for tuberculosis detection.

No	Study	Country	LoE	Sample	Comparison	Findings
12.	Kumar P *et al*., 2014 [77]	India	2b	118 TB suspected and 31 non-TB patients	in-house mPCR, MGIT culture, and smear microscopy; bacteriologically examination as reference (smear or MGIT culture)	LAMP has 100% sensitivity concerning bacteriologically, mPCR positive pulmonary, and extrapulmonary TB. Overall specificity was 93.5%.
13.	Reddy S *et al*., 2017 [78]	South Africa	2b	705 sputum samples from patients with pulmonary TB symptoms.	Smear microscopy, Xpert test; culture as a reference	LAMP sensitivity and specificity were 72.6% and 96.8%, respectively. For Smear, they were 45.4% and 98.7%. For Xpert MTB/RIF, they were 76% and 92.6%.
14.	Perera SU *et al*., 2018 [79]	Sri Lanka	2b	46 cultures of clinical samples from suspected TB patients, 31 are *M. tuberculosis*, and the rest are mycobacteria other than tuberculosis	Comparison only to culture as a reference	Sensitivity was 100%, and specificity was 66.67%
15.	Ou X *et al*., 2013 [61]	China	2b	1,378 sample set of 3 sputum samples (spot, night, and morning) from TB suspected patients	PURE-LAMP was used. Culture and ZN-smear microscopy as reference	Overall sensitivity and specificity from all three samples was 88.8% and 96.86%
16.	Bojang AL *et al*., 2013 [80]	Gambia	2b	285 sputum samples from subjects with symptoms suggestive of TB.	GeneXpert; MGIT culture as a reference	Overall sensitivity for LAMP and GeneXpert was 98.6% and 99.1%; specificity was 99% and 96%
17.	Kim CK *et al*., 2018 [81]	Korea	2b	290 sputum samples.	By the measure of agreement with rt-PCR; culture as a reference	Sensitivity was 83.6%, and specificity was 100%. Agreement with rt-PCR was 93.8%, κ=0.86.
18.	Nakiyingi L *et al*., 2018 [82]	Uganda	2b	223 sputum samples from presumptive TB patients.	Xpert MTB/RIF, FM/ZN smear; culture as a reference.	The sensitivity of LAMP, FM/ZN smear, and Xpert were 55.4%, 45.8%, and 65.1%; specificity was 98%, 96.7%, and 92%.
19.	Wang Z *et al*., 2019 [83]	China	2b	523 sputum samples from presumptive pulmonary TB patients.	Smear and culture as a reference.	Sensitivity for smear-positive/culture-positive. Smear negative/culture-positive, and culture-positive were 98.6%, 67.7%, and 89.2% respectively. Specificity was 97.5%
20.	Gray CM *et al*., 2016 [84]	India, Uganda, Peru	2b	1777 sputum samples from patients aged 18 years and above with symptoms suggestive of pulmonary TB	Smear and culture as a reference.	Overall sensitivity was 84.4%, specificity was 96.6%
21.	Thapa J *et al*., 2019 [85]	Nepal	3b	69 sputum samples from suspected TB patients.	LAMP was utilized with visual methyl green. Culture and microscopy as reference.	Sensitivity was 92.8%, specificity was 96.3% about culture, they were 92.3% and 94.6% respectively about microscopy.
22.	Cao D *et al*., 2015 [86]	China	3b	123 sputum specimens.	q-PCR. The positive case was defined using smear or culture or pulmonary imaging or lung pathology	The sensitivity of LAMP and q-PCR was 98% and 96%, respectively, while their specificity was 78.3% and 82.6%, respectively.
23.	Dayal R *et al*., 2020 [87]	India	3b	114 clinical specimens (sputum or gastric aspirate) from 114 patients aged 14 years old or less suspected of pulmonary TB.	AFB microscopy, CB-NAAT, 2 LAMP primers (IS6110 and mpb64); Culture as a reference; also uses CB-NAAT as reference	Compared to culture, LAMP 1S6110 has sensitivity and specificity of 89.8% and 60%, respectively, while mpb64 was 94.9% and 54.6%; for CB-NAAT, it was 83.1% and 69.1%, and for AFB microscopy, it was 45.8% and 90.9%. Sensitivity for both LAMP was 100% about CB-NAAT, sensitivity for 1S6110 was 81.3%, mpb64 was 68.8%.
24.	Phetsuksiri B *et al*., 2019 [88]	Thailand	4	151 MGIT cultures positive for AFB.	By the measure of agreement with an ICT.	The agreement between LAMP and ICT was 98.68%, and Kappa was 0.83.
25.	Nagai Y *et al*., 2016 [89]	Japan	4	214 clinical isolates from patients with bacteriologically confirmed pulmonary TB.	RD207-PCR and Rv0679c-multiplex PCR as reference	About RD207-PCR, the sensitivity and specificity of LAMP were both 100%. About Rv0679c-multiplex PCR, sensitivity was 99.3%, specificity was 100%.

AFB: Acid-Fast Bacillus; AUC: Area Under The Curve; CPA: Cross-Priming Amplification; CRS: Composite Reference Standard; CB-NAAT: Cartridge Based Nucleic Acid Amplification Test; EPTB: Extrapulmonary Tuberculosis; FM: Fluorescent Microscope Staining; HIV: Human Immunodeficiency Virus; HNB-LAMP: Loop-Mediated Isothermal Amplification Assay with Hydroxynaphthol Blue; ICT: Immunochromatographic Test; IS6110: insertion sequence (IS) 6110 target gene; LAMP: Loop-Mediated Isothermal Amplification; LoE: Level of Evidence; LPA: Line Probe Assay; MGIT: Mycobacteria Growth Indicator Tube; mPCR: Multiplex Polymerase Chain Reaction; N/A: not available data; Mpb64: one of target gene; PCR: Polymerase Chain Reaction; q-PCR: Quantitative Polymerase Chain Reaction; RD207-PCR: Region of Difference 207 Polymerase Chain Reaction; rt-PCR: Real Time Polymerase Chain Reaction; SAT-TB: RNA Simultaneous Amplification and Testing Method for *Mycobacterium tuberculosis*; sdaA: L-serine dehydratase gene; TB: Tuberculosis; Xpert MTB/RIF: The Gene Xpert which can identify DNA of Mycobacterium tuberculosis (MTB) and resistance to rifampin (RIF); ZN: Ziehel-Neelsen Staining. Levels of Evidence-based on Oxford Centre for Evidence-Based Medicine, 2009; 1b (Individual RCT with narrow Confidence Interval); 2a (SR with homogeneity of cohort studies); 2b (Individual cohort study, including low-quality RCT); 3b (Individual Case-Control Study); 4 (Case-series and low-quality cohort and case-control studies).

microscopy, and pooled specificity was 97.7% to 98.1% depending on the reference standard used [20]. This trend of generally high specificity compared to sensitivity is also shared with several meta-analyses and systematic reviews succeeding it [20]. In the same year, Yan *et al*. [63] published a systematic review that shows LAMP compared to SAT and Xpert for pulmonary TB diagnosis; LAMP has better sensitivity than Xpert and better specificity than SAT. Yu G reviewed LAMP as a diagnostic tool for extrapulmonary TB and shows higher specificity than sensitivity (99% and 77%, respectively) [66]. An abundance of other sources commending LAMP as an efficient and effective alternative for TB screening is represented in Table 2. We found seven articles with a level of evidence 1b, from which two studies found 100% specificity for LAMP with overall lower sensitivity, but one study shows 100% sensitivity. Another notable find is that LAMP also works well on extrapulmonary TB and pulmonary TB amongst the pediatric population. Lastly, although we managed to find three meta-analyses on LAMP as TB diagnostic tool, we have yet to find any level-1 evidence, as the available meta-analysis uses observational studies [66–68].

Compared to real-time PCR (rtPCR), LAMP has a lower sensitivity (91.3% and 89.13% respectively). However, LAMP remains advantageous in other aspects. The time taken for the LAMP procedure is considerably less than PCR, taking only 100 minutes in the entire process. LAMP is also cheaper because it only needs a water bath with a constant heat of 60-65 °C instead of a thermal cycler. The efficiency is especially appealing to developing countries with scarce resources and equipment [19,69–71].

Regardless of its advantages, LAMP still struggles to replace current molecular diagnostic tools. Xpert MTB/RIF, though relatively more costly, has been trusted to detect antibiotic resistance. The same cannot be said for LAMP, thus WHO recommendations note that implementation of LAMP, while it may entirely or partially replace conventional sputum microscopy, should not replace Xpert MTB/RIF [14,20]. Though, following the study by Takarada *et al*. [18], WHO may revise said recommendation when more studies assess the accuracy of the proposed method.

In exploring the advantages and applications, although studies on LAMP and Au-Np combination are scarce, the research by Kaewphinit *et al*. [15] has at least shown that LAMP and Au-Np combination can be implemented for rapid detection of TB in a developing country, in this case, Thailand. On the other hand, studies using LAMP without Au-Np as a diagnostic tool are abundant and show the applicability of LAMP in different countries of different conditions. In several multicenter studies, LAMP is applicable in China [42,61,63,66,83,86], India [74,77,84,87], Sri Lanka [79], Nepal [85], Thailand [12,15,72,88], Vietnam [70,76], Indonesia [90], Malawi [71], South Africa [76,78], Ethiopia [75], Gambia [80], Zambia [73], Uganda [82,84], Peru [76,84], Brazil [76], Japan [68,89], and Korea [81]. Most studies about LAMP were carried out in developing countries that aim to solve the diagnostic problem of TB in burdened countries. A look into the Indonesia study, carried out by Lisdawati *et al*. [90], stated that LAMP efficiently produces up to 30 clinical isolates in two hours with a 94.2% positivity rate. As LAMP performance in the studies shows promise, it could be said that LAMP is suitable in even resource-limited areas, and it is also possible to implement the novel LAMP Au-Np in rural areas.

Current limitations and moving forward

Notably, the current review contains only two studies about using LAMP and Au-Np specifically for rifampicin and isoniazid resistance detection [12,13,15]. Even then, said literature uses other commercial molecular diagnostic tools as reference standards. In this review, most studies come from developing countries and mainly in Asia, where TB is endemic and problematic due to the relatively large population. Thus, there is a necessity for repeat and validating studies on this matter. Through independent research to prove LAMP and Au-Np's effectivity for detecting TB, with the excellent reference standard, the right direction for future study is to assess the accuracy of TB diagnosis and resistance detection LAMP and Au-Np combination. Another lacking aspect is the cost and time-to-result profile of the LAMP and Au-Np combination as a whole. Though an estimated 75 minutes was stated in one study, other studies have yet to corroborate that fact. Finally, since the goal is to find a more applicable method for resistance detection, studies should also examine the tool's performance in the resource-limited area.

Conclusions

The ever-increasing drug resistance cases in TB have created an urgency to develop a reliable, cost-effective, and applicable diagnostic method. One such method is the LAMP and Au-Np combination, which has so far shown a shorter time to get results and impressive accuracy. LAMP – Au-Np should also be

relatively cheaper because it does not need a high temperature to work and requires less equipment.

As a standalone diagnostic tool, both Au-Np and LAMP appear to have good accuracy in detecting the presence of *M.tuberculosis* infection. However, even though we found a few studies stating LAMP and Au-Np combination have 100% sensitivity and specificity for the detection of antibiotic resistance, we have yet to see any research that also says the result's confidence interval. Additionally, a more in-depth analysis of LAMP and Au-Np combinations – such as with meta-analysis – should be performed before applying this method.

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All authors of this paper have substantial contributions in conceiving and designing the paper; attaining, analyzing, and interpreting paper data to create a review; drafting the paper itself or revising it significantly; and agreed to publish and accepted its responsibilities as an author. *Conceptualization*: Habiburrahman M; *Methodology*: Habiburrahman M, Ariq H; *Formal analysis*: Habiburrahman M, Ariq H, Handayani RRD; *Data curation*: Habiburrahman M, Ariq H, Handayani RRD. *Software*: Habiburrahman M; *Validation*: Habiburrahman M, Ariq H, Handayani RRD; *Investigation*: Habiburrahman M, Ariq H, Handayani RRD; *Writing* - original draft preparation: Habiburrahman M, Ariq H; *Writing - review and editing*: Habiburrahman M, Handayani RRD; *Approval of final manuscript*: all authors.

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