TB-LAMP

Implementation Experiences and Lessons Learned

Stop TB Partnership

BACKGROUND

The Loopamp™ MTBC assay (Eiken Chemical Company Ltd, Tokyo, Japan) is a rapid molecular diagnostic that uses the loop-mediated isothermal amplification (LAMP) technique for the detection of tuberculosis (TB). This assay is commonly known as TB-LAMP. In 2016, based on a systematic review and meta-analysis of data from 20 studies conducted in 17 countries, the World Health Organization (WHO) recommended the use of TB-LAMP as a replacement for sputum smear microscopy.[1]

TB-LAMP's minimal infrastructure requirements and simple instrumentation make it an attractive option for laboratories at the peripheral level in low-and middle-income countries, as well as for active case-finding activities that result in significant numbers of samples that can be batch tested using the HumaLoop T instrument (up to 70 samples per 8-hour shift). The price of $6.00[2] per sample when batching optimally means TB-LAMP is currently the only WHO-recommended rapid molecular diagnostic that meets the target price established by WHO for a replacement test for microscopy.[3]

Although TB-LAMP is a sensitive tool for TB diagnosis, it has not been widely used since the WHO recommendation in 2016. One reason for this is that TB-LAMP cannot detect rifampicin resistance, so many countries have instead focused on adoption of other rapid molecular diagnostics. In remarks accompanying its recommendation, the WHO indicated that TB-LAMP should not replace the use of rapid molecular diagnostics that detect TB and resistance to rifampicin, especially among populations at risk of MDR-TB.

A growing number of countries have piloted or rolled out TB-LAMP in recent years, and the Stop TB Partnership sought to better understand how different countries have implemented the test and how the end users perceive it. To achieve this, national TB program (NTP) managers, national TB reference laboratory managers and implementing partners in four countries using TB-LAMP were surveyed on their experiences. By documenting and compiling these experiences, other countries may better understand the benefits of the TB-LAMP test, as well as its limitations, and how it could fit within diagnostic networks among other WHO-recommended rapid molecular diagnostics, including GeneXpert and Truenat.

1. *WHO consolidated guidelines on tuberculosis: module 3: diagnosis: rapid diagnostics for tuberculosis detection, 2021 update. https://www.who.int/publications/i/item/9789240029415*
2. *Stop TB Partnership GDF technical info. Note – TB LAMP (2020). https://www.stoptb.org/gdf-technical-info-note-tb-lamp*
3. *WHO meeting report: High-priority target product profiles for tuberculosis diagnostics: report of a consensus meeting (2014). https://apps.who.int/iris/bitstream/handle/10665/135617/WHO_HTM_TB_2014.18_eng.pdf?sequence=1*

Diagnostic performance

TB-LAMP is a manual molecular test with two amplification targets (*IS6110* and *gyrB*). The test has performance comparable to that of Xpert MTB/RIF, with a sensitivity of 77.7% (95% CI: 71.2–83.0) and specificity of 98.1% (95% CI: 95.7–99.2) compared to a culture reference standard.[1]

At the time of the WHO review, there was limited evidence available on the performance of TB-LAMP when testing samples from people living with HIV (PLHIV). As a result, WHO concluded that it was unclear whether TB-LAMP had additional diagnostic value over sputum smear microscopy for the testing of PLHIV with signs and symptoms consistent with pulmonary TB.[1] However, additional evidence that has become available since the initial WHO review shows the effectiveness of TB-LAMP in PLHIV, although the sensitivity is lower than that of Xpert MTB/RIF (**SEE TABLE 1**).[4,5,6]

A study conducted among PLHIV who were not receiving antiretroviral therapy in South Africa found that TB-LAMP demonstrated a sensitivity of 63.0% (95% CI: 49.0–75.0) and specificity of 98.5% (95% CI: 97.0–99.2) compared to Mycobacteria Growth Indicator Tube (MGIT) culture as the reference standard. When compared to Xpert MTB/RIF, the sensitivity of TB-LAMP was 67.2% (95% CI: 53.5–78.6) and specificity 99.0% (95% CI: 97.8–99.5). The sensitivity of TB-LAMP was lower than that of Xpert MTB/RIF, which had a sensitivity of 74.0% (60.4–84.3%) when compared to MGIT culture as the reference standard, although the specificity was comparable.[4] In a study conducted in Nigeria, TB-LAMP had a sensitivity of 60.0% (95% CI: 35.8–80.2) and specificity of 99.6% (95% CI: 98.8–99.9), when compared to Lowenstein-Jensen (LJ) culture, with a similar performance as Xpert MTB/RIF (sensitivity of 66.7%; 95% CI: 41.7–84.8).[5] A smaller study of PLHIV in Uganda (113/233) demonstrated a sensitivity of 52.3% when using LJ and/or MGIT culture as the reference standard.[6]

While there is limited evidence on the performance of TB-LAMP in pediatric populations, a study conducted in Thailand demonstrated a sensitivity of 76.5% (95% CI: 50.1–93.2) when compared to MGIT culture as the reference standard. Further research is needed in high-burden settings to determine the effectiveness of TB-LAMP as a diagnostic tool for pediatric TB.

TABLE 1

DIAGNOSTIC PERFORMANCE OF TB-LAMP AMONG PLHIV

Country	Number of PLHIV in study	Reference	Sensitivity	Specificity
South Africa[4]	**783** (ART clinic: all PLHIV)	MGIT culture	**63.0%** (49.0 - 75.0)	**98.5%** (97.0 - 99.2)
		Xpert MTB/RIF	**67.2%** (53.5 - 78.6)	**99.0%** (97.8 - 99.5)
Nigeria[5]	**1,280 of 2,636** (48.6% PLHIV)	LJ culture	**60.0%** (35.8 - 80.2)	**99.6%** (98.8 - 99.9)
Uganda[6]	**113 of 233** (48.5% PLHIV)	LJ and/or MGIT culture	**52.3%** (36.7 - 67.5)	**97.1%** (89.9 - 99.6)
		LJ and/or MGIT culture and/or Xpert MTB/RIF	**48.0%** (33.7 - 62.6)	**98.4%** (91.5 -100)

1. *WHO consolidated guidelines on tuberculosis: module 3: diagnosis: rapid diagnostics for tuberculosis detection, 2021 update.* https://www.who.int/publications/i/item/9789240029415
4. *Spooner E., Reddy S., Ntoyanto S., Sakadavan Y., Reddy T., Mahomed S. et al (2022). TB testing in HIV-positive patients prior to antiretroviral treatment. Int J Tuberc Lung Dis.* Doi: 10.5588/ijtld.21.0195
5. *Odume B., Nwokoye N., Spruijt I., Slyzkyi A., Dim C., Chukwuog O. et al (2021). Diagnostic accuracy of TB-LAMP for the diagnosis of pulmonary tuberculosis among adult presumptive TB in Nigeria.* GJMS: 11(2):122-291
6. *Nakiyingi L., Nakanwagi P., Briggs J., Agaba T., Mubiru F., Mugenyi M. et al (2018). Performance of loop-mediated isothermal amplification assay in the diagnosis of pulmonary tuberculosis in a high prevalence TB/HIV rural setting in Uganda. BMC Infect Dis.* Doi: 10.1186/s12879-018-2992-1

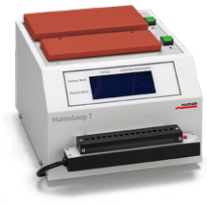
Instrumentation and infrastructure requirements

The TB-LAMP assay requires proprietary reagents for DNA extraction and TB detection, wide-bore pipette tips for pipetting sputum samples, and the HumaLoop T instrument (**SEE ANNEX 1** for pricing), which serves as a heat block for the sample inactivation and amplification phases of the assay and has a blue LED light for interpretation of test results with the naked eye (**FIGURE 1**). A benchtop microcentrifuge, HuMax ITA, with pre-configured programs for incubation, mixing, and centrifugation of Loopamp reaction tubes can be purchased as an optional extra. TB-LAMP has minimal infrastructure requirements and biosafety precautions similar to those needed for sputum smear microscopy.[1] Although an air-conditioned room is not mandatory to perform the test, the recommended maximum operating temperature for the TB-LAMP test is 30°C. Additionally, while the test kits can be stored within the temperature range of 2–30°C, they should be refrigerated at 2–8°C if the storage room temperatures exceed 30°C.[7] Nonetheless, Eiken has conducted temperature tolerance tests and shown that the TB-LAMP test can be performed between 30–40°C without problems.

While brief power cuts should not affect the temperature of the HumaLoop T instrument, a UPS and battery pack can be purchased locally to allow the use of the instrument in settings with longer power cuts.

FIGURE 1

INSTRUMENT, KITS AND CONSUMABLES NEEDED TO RUN THE TB-LAMP ASSAY AVAILABLE IN THE STOP TB PARTNERSHIP'S GLOBAL DRUG FACILITY (GDF) CATALOG THROUGH THE GLOBAL DISTRIBUTOR FOR TB-LAMP, HUMAN DIAGNOSTICS[2]



HumaLoop T

Loopamp™ PURE DNA Extraction Kit

Loopamp™ MTBC Detection Kit

Pipette-60 Set

IMAGES: FROM HUMAN DIAGNOSTICS

1. *WHO consolidated guidelines on tuberculosis: module 3: diagnosis: rapid diagnostics for tuberculosis detection, 2021 update.* https://www.who.int/publications/i/item/9789240029415
2. *Stop TB Partnership GDF technical info. Note – TB LAMP (2020).* https://www.stoptb.org/gdf-technical-info-note-tb-lamp
7. *Frequently asked questions – TB LAMP.* https://human.de/04_Molecular_DX/Isothermal_Amplification/Marketing%20Material/981017-3_FAQ_TB-LAMP_2020-09.pdf

TB-LAMP procedure

The TB-LAMP test includes a closed tube system that reduces the risk of contamination. Testing steps (**SEE FIGURE 3**) include manual ultra-rapid DNA extraction followed by amplification on the HumaLoop T instrument, with a positive and negative control included with each cycle. Briefly, 60µl of sputum is added to a heating tube containing sodium hydroxide (NaOH) extraction solution and heated at 90°C for 5 minutes on the HumaLoop T heating unit to break open and inactivate the TB bacteria. DNA is then purified using the DNA extraction kit[8] and approximately 30µl of the extracted DNA is transferred to the LAMP reaction tube (containing dried primers and buffer). The LAMP reaction tube is then placed on the HumaLoop T reaction unit for amplification at 67°C for 40 minutes.[9] The reaction proceeds until large amounts of DNA are generated, and this can be visualized by using a dye molecule that fluoresces under blue LED light. The entire sample processing and testing is complete in about 1.5 hours. Up to 70 samples can be processed per working day by running 5 cycles of 14 samples per batch.

Eiken currently offers Loopamp assays for TB and malaria (Pan/Pv/Pf). For TB-LAMP, the HumaLoop T instrument is used while for malaria, a separate instrument, HumaLoop M, with heating block temperatures set at 75°C and 65°C for the extraction and detection steps, is used. Due to the pre-configured parameters of the HumaLoop T instrument, multi-disease testing is not possible. However, Eiken has an alternative system called HumaTurb as a solution for reference or regional laboratories, which allows for multi-disease testing and can process up to 96 samples per run. The HumaTurb system allows for real-time turbidity measurements based on magnesium-pyrophosphate generation during the amplification process. A photometer detects the absorbance every six seconds, and the results are interpreted based on correlation with absorbances obtained from the negative and positive controls. The system comes with a built-in printer and allows for automatic data transfer. It consists of two parts, the control unit (HumaTurb C) for incubation and temperature control, and the amplification unit (HumaTurb A). Each HumaTurb A unit contains two independent reaction blocks with a capacity of 8 tests on each, meaning if 6 HumaTurb A units are connected, 96 samples can be processed in a run. This means two different disease assays can be performed in one HumaTurb A unit simultaneously. Currently, the default settings are for TB and malaria amplification. The assays for leishmaniasis, human African trypanosoma, and Chagas disease can be used for research purposes. Note that WHO has not assessed the performance of the Loopamp MTBC assay on the HumaTurb system.

FIGURE 2

THE HUMATURB SYSTEM

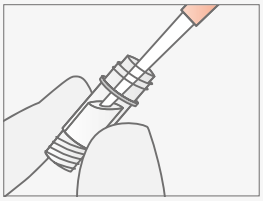
IMAGE: FROM HUMAN DIAGNOSTICS

8. *Instructions for Use. Loopamp PURE DNA extraction kit. https://www.eiken.co.jp/uploads/IFU/380237-C_ en_ 20220301.pdf*
9. *Instructions for Use: Loopamp MTBC detection kit. https://www.eiken.co.jp/uploads/Loopamp%20MTBC%20detection%20kit.pdf*

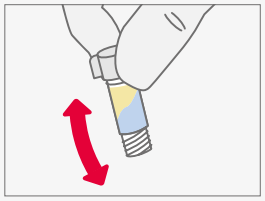
FIGURE 3

TB-LAMP PROCEDURE

Sample transfer and lysis



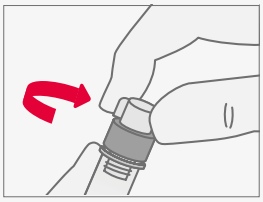
Transfer 60 µl sputum with Pipette-60 into the heating tube.



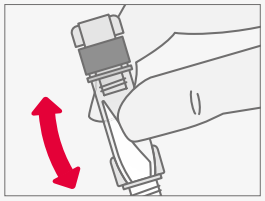
Mix well the contents of the tube by shaking.

Incubate the tube in the HumaLoop T heating unit for 5 min at 90°C.

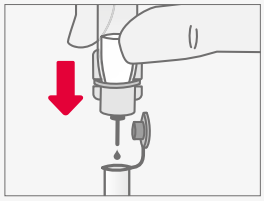
Loopamp™ PURE DNA extraction



Screw the heating tube onto the adsorbent tube.

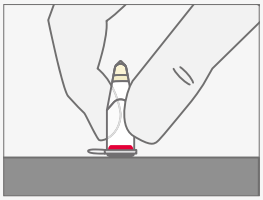


Shake the tube until a milky solution is obtained.

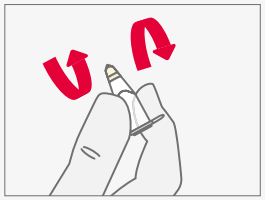


Screw the injection cap onto the adsorbent tube.
Extract 30 µl of the DNA into the reaction tube.

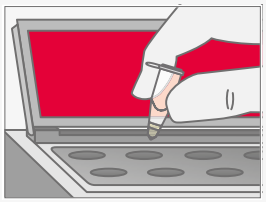
Loopmediated isothermal amplification



Incubate the tube upside down for 2 min. at room temperature to reconstitute the reagents in the cap.

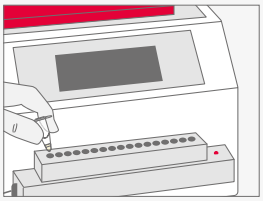


Mix the tube 5 times and tap the reaction tube point down on a hard surface until the reaction mix is collected at the bottom.

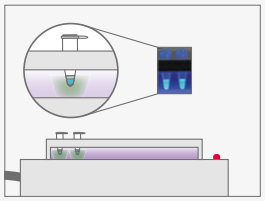


Incubate the reaction tube at 67°C for 40 min in the reaction unit.

Result reading



Insert the tube into the detection unit and turn on the UV light.



Positive results fluoresce green, negative results show no fluorescence.

SOURCE: REPRODUCED FROM HUMAN DIAGNOSTICS
https://www.human.de/04_Molecular_DX/Isothermal_Amplification/Marketing%20Material/981014_Flyer_TB-LAMP_EN.PDF

IMPLEMENTATION EXPERIENCE OF SURVEYED COUNTRIES USING TB-LAMP

The instrument install base across the four countries surveyed ranged from 11 in the Philippines to 50 in Cameroon (**TABLE 2**). TB-LAMP testing was generally implemented in locations where Xpert testing was not feasible due to infrastructure constraints or where high-throughput testing was needed during screening campaigns. The lessons learned from the implementation in different countries are provided in **TABLE 4** at the end of this document.

TABLE 2

SUMMARY OF TB-LAMP PLACEMENT, USE AND PLANS FOR SCALE-UP

Country	Number of instruments	Instrument placement and use	Plans for scale-up
Cameroon	50	National/referral laboratory, district and peripheral level for programmatic use	Planning for 65 instruments across the country by 2026. Have also supported implementation efforts in other countries.
Nigeria	42	District level for programmatic use and mobile testing	11 additional instruments are expected through USAID support.
Zambia	26	Peripheral facility for programmatic use and mobile testing	Plan to install at least 2 instruments in each province to replace smear microscopy at facilities without Xpert.
The Philippines	11	National/referral laboratory, district and peripheral level for a pilot project	Instruments were donated to the NTP at the end of projects with no further funding for reagents. No plans for scale-up.

Cameroon

This country was one of the first to implement TB-LAMP starting in 2017, evaluating its effectiveness in detecting TB in prisons and active case finding in 2018 and 2019. Evaluations were also conducted in PLHIV and children and with extrapulmonary samples from 2019 to 2022 (data still under review for publication; expected in 2023). When TB-LAMP was used first followed by Xpert MTB/RIF for rifampicin resistance detection on samples found to be MTB positive on TB-LAMP, an analysis of mass screening campaigns in prisons and villages showed a potential reduction of over 70% in the time to get results and a cost savings of $16,934 in prisons and $1,377 in villages, in years two and three compared to a strategy of testing with Xpert MTB/RIF alone.[10] This faster testing time can help people with TB get treatment earlier. TB-LAMP is especially beneficial in mass screening campaigns where sample volumes are high; diagnostic facilities with the four-module GeneXpert instruments can generally only test up to 16 to 20 samples per 8-hour working day. Cameroon also implemented the use of TB-LAMP as a follow-on test to smear microscopy for smear-negative sputum specimens from adults with signs and symptoms consistent with pulmonary TB. This activity was done through an organized sample transportation system for the transportation of smear-negative sputum specimens to TB-LAMP sites. In 2019, this activity helped to detect up to 388 (14.8%) individuals with TB out of 3,061 smear-negative sputum specimens transferred to TB-LAMP sites.[11]

One disadvantage of the TB-LAMP assay is that it does not produce digital data, therefore, the results cannot be automatically transmitted to clinicians or electronic patient registers. The Cameroon NTP is currently piloting the DataToCare mobile application developed by Savics (Brussels, Belgium) to send results directly to clinicians by SMS or email and generate reports (**FIGURE 4**). The test information is entered manually by the operator and transferred to a server where the NTP can visualize trends in utilization and generate reports using the DataToCare dashboards. The test data is linked to the patient management database, allowing for visualization of the diagnostic cascade.

FIGURE 4

DATATOCARE SET-UP IN CAMEROON INTEGRATING GENEXPERT, MICROSCOPY AND TB-LAMP RESULTS

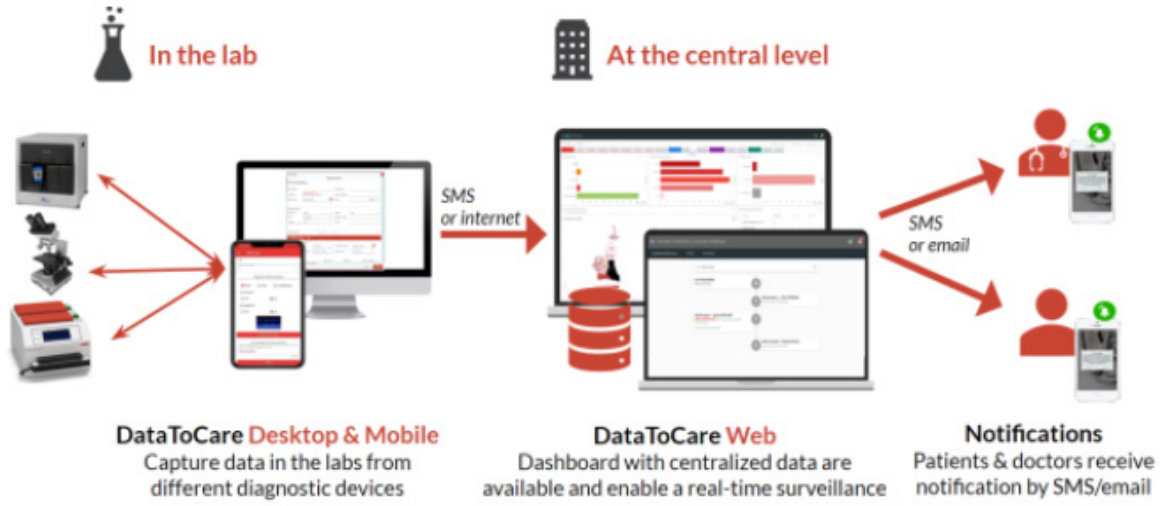


IMAGE: PROVIDED BY THE NATIONAL TUBERCULOSIS CONTROL PROGRAM, CAMEROON

10. *Donkeng-Donfack VF., Tchatchueng-Mbougua JB., Abanda NN., Ongoulal SM., Djieugoue YJ.et al (2022). A cost-benefit algorithm for rapid diagnosis of tuberculosis and rifampicin resistance detection during mass screening campaigns. BMC Infect Dis. Doi: 10.1186/s12879-022-07157-0*

11. *Donkeng-Donfack VF., Ongoulal SM., Djieugoue YJ., Simo YK., Manga H., Tollo DAD., et al (2022). Tuberculosis-loop-mediated isothermal amplification implementation in Cameroon: Challenges, lessons learned and recommendations. Afr J Lab Med. Doi: 10.4102/ajlm.v11i1.1792*

IMAGE SOURCE: KNCV NIGERIA

Nigeria

KNCV TB Foundation Nigeria, a USAID-funded TB Local Organization Network (LON) partner, has led the introduction of TB-LAMP testing in Nigeria. A pilot study was conducted to evaluate the accuracy of the test in comparison to solid culture and demonstrated a sensitivity of 76.7% (95% CI: 68.8-83.2) and specificity of 99.3% (95% CI: 98.9-99.6).[12] As described earlier, among PLHIV, TB-LAMP was found to have a sensitivity and specificity similar to the performance of Xpert MTB/RIF. These results paved the way for the adoption of TB-LAMP into the national guidelines as a replacement for smear microscopy in settings where Xpert is not available.

Currently, TB-LAMP instruments are installed in six of the fourteen states at tertiary and secondary/district levels, but none at the primary/peripheral level. When sputum samples are received for TB-LAMP testing, an aliquot is taken for the test, and the sample is also reserved for Xpert testing. If the sample tests are TB positive on TB-LAMP, the reserved sample is sent to an Xpert testing site to determine rifampicin susceptibility.

TB-LAMP is utilized for active case finding in an innovation called Wellness of Keke (Wok), which provides a one-stop mobile shop using a motorized tricycle for TB screening and diagnosis (ultraportable chest X-rays with artificial intelligence-powered software for the computer-aided detection of TB, together with TB-LAMP), chronic disease screening (including diabetes and hypertension) and COVID-19 vaccination for underserved and hard-to-reach areas. The HumaLoop TB instrument is powered by an external battery source run using a portable solar panel provided by the manufacturer. There is also consideration being given to connecting the solar system to the tricycle. The KNCV team also tested the use of Truenat MTB Plus and MTB-RIF Dx tests (Molbio Diagnostics, India) in this "Wok" innovation but found that the throughput was much lower compared with TB-LAMP. Although Truenat tests provided the advantage of on-site rifampicin testing, same day testing was not always feasible when dealing with large sample volumes. Additionally, when used at maximum efficiency with 14 samples in a batch, the TB-LAMP test is less expensive than the Truenat test ($6.00 vs $7.90 respectively, however TB-LAMP positive tests need to be referred for rifampicin resistance testing).

12. *Odume B., Nwokoye N., Spruijt I., Slyzkyi A., Dim C., Chukwuog O. et al (2021). Diagnostic accuracy of TB-LAMP for the diagnosis of pulmonary tuberculosis among adult presumptive TB in Nigeria. GJMS: 11(2):122-291*

Zambia

In an initial study at 3 hospital laboratories in 2018, TB-LAMP had a higher positivity rate (22.4%) compared to smear microscopy (14.6%) when testing people with signs and symptoms of TB.[13] With the NTP continuing to expand the use of GeneXpert in parallel, this provided an opportunity to identify sites that could benefit from TB-LAMP as a complementary and synergistic approach to rapid molecular diagnosis, allowing for diagnosis at lower tiers of the health system. Samples that tested positive using TB-LAMP were referred to GeneXpert sites for rifampicin resistance testing using established sample referral networks. The NTP aims to phase out the use of smear microscopy by placing TB-LAMP in facilities without GeneXpert, with initial plans to have at least 2 TB-LAMP testing sites at the peripheral level in each province.

However, to ensure cost-effectiveness, it's important to optimize the sample referral network. The ideal batch size for TB-LAMP testing is 14 samples per batch as smaller batch sizes significantly increase the cost per test. This is because controls are required for each run, and testing only a few samples at a time increases the cost. For example, the cost per test goes up to $9.38 if only 2 samples are tested per batch, compared to $6.00 if 14 samples are tested (**SEE ANNEX 2**). To maximize cost and efficiency at least approximately 7 samples should be processed per batch as the cost difference becomes minimal around this point.

13. *Solo ES., Mbulo G., Lungu PS., Nsama D., Zulu FM, Muzyamba J. et al (2019). Evaluation of TB-LAMP assay for the diagnosis of pulmonary tuberculosis in Lusaka, Zambia. Clin Sc Res Rep. Doi: 10.15761/CSRR.1000126*

The Philippines

Prior to implementation, a validation study was conducted in 2017, initially at the reference laboratory (n=279) and then at 5 peripheral laboratories (n=507), with results showing higher sensitivity than smear microscopy and comparable sensitivity to Xpert MTB/RIF (**TABLE 3**). At the time of the evaluation, microscopy was still the main diagnostic tool and Xpert MTB/RIF was used only for patient groups at high risk of drug resistant TB. The TB-LAMP instruments were used in two projects, USAID's TB Innovations and Health Systems Strengthening Project (TBIHSS) and a Japan government-supported JICA (Japan International Cooperation Agency) project, for intensified active case-finding activities and aiding diagnosis in private laboratories and high-volume sites. Positive TB-LAMP results were subsequently tested with Xpert MTB/RIF for rifampicin resistance detection by referral to the nearest GeneXpert laboratory. The TBIHSS project was affected by COVID-19 lockdowns and had to stop earlier than planned, but the highest number of TB-LAMP tests were conducted at a health facility using the test for both passive and active case-finding activities, accounting for 326 of the 448 tests conducted under the project.

TB-LAMP was incorporated into the national guidelines in 2020[14] for use in facilities where Xpert testing is not available. Individuals who test positive for TB-LAMP are then tested with Xpert MTB/RIF to detect rifampicin resistance. Those that have a positive result on TB-LAMP but a negative result on Xpert MTB/RIF are considered bacteriologically confirmed drug-susceptible TB. TB-LAMP allowed for the testing of a larger number of samples than Xpert MTB/RIF in a given period, particularly during active case finding, which enabled results to be issued on the same day as testing. However, there are currently no plans for scale-up of TB-LAMP testing as the focus is being placed on expanding rapid molecular diagnostics that can also test for rifampicin resistance.

TABLE 3

DIAGNOSTIC PERFORMANCE OF TB-LAMP AND XPERT MTB/RIF IN THE PHILIPPINES COMPARED TO MGIT CULTURE

Diagnostic test		Phase 1 (Central level, n = 279)	Phase 2 (Peripheral level, n = 507)
TB-LAMP	**Sensitivity**	86.1 (95% CI: 78.7 - 93.4)	73.5 (95% CI: 66.1 - 80.0)
	Specificity	95.7 (95% CI: 92.9 - 98.6)	97.1 (95% CI: 94.7 - 98.6)
Xpert MTB/RIF*	**Sensitivity**	93.0 (95% CI: 87.6 - 98.4)	65.7 (95% CI: 55.6 - 74.8)
	Specificity	95.8 (95% CI: 92.9 - 98.7)	95.6 (95% CI: 92.8 - 97.5)

*N= 276 IN PHASE 1 AND 440 IN PHASE 2

14. *NTP manual of procedures 6th edition (2020). https://ntp.doh.gov.ph/download/ntp-mop-6th-edition/#*

LESSONS LEARNED

Training requirements

The TB-LAMP test has minimal training requirements and is intended for use by users with minimal knowledge of molecular biology.
A 2-day training was described as adequate to ensure competency in the manual steps and use of instrumentation.

Sample referral system

Samples that test positive using TB-LAMP need to be rapidly referred for a rifampicin resistance test. There is therefore a need for a well-designed and functioning sample referral system to ensure rifampicin resistance testing and return of results is timely.

Instruments and consumables

The instruments are easy to install without the need for special laboratory rooms or air-conditioning. The same rooms used for smear microscopy were used for TB-LAMP without modifications.

The instruments do not take up a lot of space.

TB-LAMP is well suited for places with erratic power supply, and when coupled with solar panels can allow uninterrupted testing during longer power outages.

The frequency of errors/invalids using TB-LAMP is much lower than the frequencies observed with other rapid molecular diagnostics: across study settings and countries, the error/ invalid rate has been reported to be below 0.1%.[15,16]

When processing fewer than 14 tests in a run, there is a wastage of reagents and controls owing to the need for controls in every run. There is therefore a need to carefully plan the ordering and testing to avoid stock-outs of some components. Furthermore, the cost per test increases when batching is not done (**SEE ANNEX 2**).

Instruments are robust but require cleaning (to remove dust) and covering after use. In Cameroon and Nigeria, each TB-LAMP testing laboratory has maintenance sheets where the daily, weekly and monthly maintenance to clean machine parts is completed. The local distributor completes the annual maintenance to check and adjust the fluorescence LED lights and check the temperature of the reaction block for amplification and detection.

15. *Donkeng-Donfack VF., Ngando L., Yone-Pefura EW., Che DS., Ateba G. et al (2018). Comparative study of Loopamp Mycobacterium tuberculosis Complex kit for rapid detection of Mycobacterium tuberculosis complex in Cameroon. BBRJ.2:1:46-52*

16. *Gray CM., Katamba A., Narang P., Giraldo J., Zamudio C., Joloba M. (2016). Feasibility and Operational Performance of Tuberculosis Detection by Loop-Mediated Isothermal Amplification Platform in decentralized settings: Results from a Multicenter Study. J Clin Microbiol. 10.1128/JCM.03036-15*

Use in active case finding

The TB-LAMP test is well suited for active case finding where the sample volume is high because 14 samples can be tested within 1.5 hours.

An algorithm based on initial testing with TB-LAMP followed by Xpert may allow for early and rapid TB diagnosis while reducing the cost.[10]

In Nigeria, TB-LAMP is used in active case finding coupled with X-ray/CAD.

User perspectives

The TB-LAMP procedure is simple and user-friendly, taking less time and effort than smear microscopy.

The hands-on time is under 50 minutes, similar to that of smear microscopy.

The number of tests able to be done per day with TB-LAMP is more than with Xpert or Truenat, allowing results to be released on the same day of testing when there are large volumes of samples.

The test is less affected by environmental temperature than Xpert. It can therefore be implemented in a remote laboratory.

The inability of TB-LAMP to test for rifampicin resistance is a major disadvantage.

Results are not stored on the instrument, which is a disadvantage.

TB-LAMP entails several manual steps and requires training to ensure user proficiency.

10. *Donkeng-Donfack VF., Tchatchueng-Mbougua JB., Abanda NN., Ongoulal SM., Djieugoue YJ.et al (2022). A cost-benefit algorithm for rapid diagnosis of tuberculosis and rifampicin resistance detection during mass screening campaigns. BMC Infect Dis. Doi: 10.1186/s12879-022-07157-0*

COMPARISON OF COSTS AND OPERATIONAL CHARACTERISTICS OF TB-LAMP AND OTHER RAPID MOLECULAR TESTS

A key consideration in implementing rapid molecular tests is the cost. A comprehensive comparison of cost-effectiveness of tools should take into consideration country-specific costs including human resources, specimen referral for rifampicin testing, and other implementation components. **SEE TABLE 4** for a comparison of procurement costs and operational characteristics of TB-LAMP, Truenat and Xpert.

TABLE 4

PROCUREMENT COSTS AND OPERATIONAL CHARACTERISTICS OF TB-LAMP, TRUENAT AND XPERT TESTS

Category	TB-LAMP	Truenat	Xpert MTB/RIF or Ultra
Infrastructure requirements	Auxiliary batteries may be required in settings that experience longer blackouts. Air-conditioner may be required for reagent storage if ambient temperatures exceed 30°C	Surge protectors recommended in settings that experience frequent voltage fluctuations. Air-conditioner may be required for reagent storage (for chips) if ambient temperatures exceed 30°C	Uninterrupted power source (UPS) required, with auxiliary batteries depending on duration of blackouts. Air-conditioner may be required for testing in settings where ambient temperatures exceed 30°C, and for reagent storage (storage under 28°C)
Operating temperature	Max 40°C	Max 40°C	Max 30°C
Throughput	14 samples/batch ~70 samples/8 hour work day	12-15 samples/8 hour work day for Truenat Duo workstation	16-20 samples/8 hour work day for GeneXpert 4-module instrument
Equipment costs[17] (US$)	$2,695 (HumaLoop T, plus optional HuMaxITA centrifuge for $632.50)	$14,000 (Truenat Duo workstation)	$19,000 (GeneXpert 4-module 10-color workstation)
Extended annual warranty costs (US$)	$1,842.50*	$1,120 (Truenat Duo workstation)	$2,898** (GeneXpert 4-module workstation)
Reagent costs per sample[17] (US$)	$6.00***	$7.90	$9.98

*Does not include costs of travel and accommodation of service provider.

**Does not include costs of travel, accommodation and labor of service provider.

*** TB-LAMP reagent cost per sample varies with batch size: from $6.00/test if 14 samples tested per batch to $13.17 if only one sample is tested in a batch (see Annex 2).

17. *Stop TB Partnership GDF Diagnostics, Medical Devices and Other Health Products Ordering List (2023). https://www.stoptb.org/global-drug-facility-gdf/gdf-product-catalog*

The scenarios presented in **TABLE 5** assume that the TB-LAMP test is placed at a peripheral-level facility, that 15% of the tests done will be positive and therefore require rifampicin resistance testing using Xpert MTB/RIF at a referral site The limitations of this basic analysis are that only reagent costs are included. More detailed analysis would need to include the number of people undergoing investigation for TB, positivity and prevalence rates, human resources, sample referral and maintenance costs. Nonetheless, this basic analysis suggests that TB-LAMP, when batched, may be a cost-effective alternative to using Xpert MTB/RIF on each sample, particularly when 3 or more samples are tested in a batch. This demonstration of cost-effectiveness however can not be extended to comparison of running Truenat on each sample, given the lower reagent cost for Truenat compared to Xpert MTB/RIF.

TABLE 5

COST-ANALYSIS OF TB-LAMP UNDER DIFFERENT TEST VOLUMES ASSUMING PLACEMENT AT A PERIPHERAL-LEVEL FACILITY WITH REFERRAL TO AN XPERT SITE FOR RIFAMPICIN RESISTANCE TESTING

Number of samples to test/day	Cost per TB-LAMP test	Total daily TB-LAMP reagent costs	Cost of rifampicin resistance testing using Xpert	Total reagent cost	Average cost/sample tested
1	$13.17	$13.17	$1.50	$14.67	**$14.67**
2	$9.31	$18.62	$2.99	$21.61	**$10.81**
3	$8.02	$24.06	$4.49	$28.55	**$9.52**
5	$7.00	$35.00	$7.49	$42.49	**$8.50**
10	$6.22	$62.20	$14.97	$77.17	**$7.72**
14	$6.00	$84.00	$20.96	$104.96	**$7.50**
28	$6.00	$168.00	$41.92	$209.92	**$7.50**

Cost per TB-LAMP test = sliding cost of TB-LAMP per number of samples tested in a batch (see Annex 2).
Total daily TB-LAMP reagent cost = cost of TB-LAMP reagents for the tests done in a day.
Cost of rifampicin resistance test using Xpert = average daily cost of Xpert testing assuming 15% of the TB-LAMP tests done per day need follow-on rifampicin resistance testing.
Total reagent cost = total cost of testing for TB and rifampicin resistance.
Average cost/sample tested = total reagent cost divided by the number of samples tested performed per day.

RECOMMENDED USE CASES OF TB-LAMP

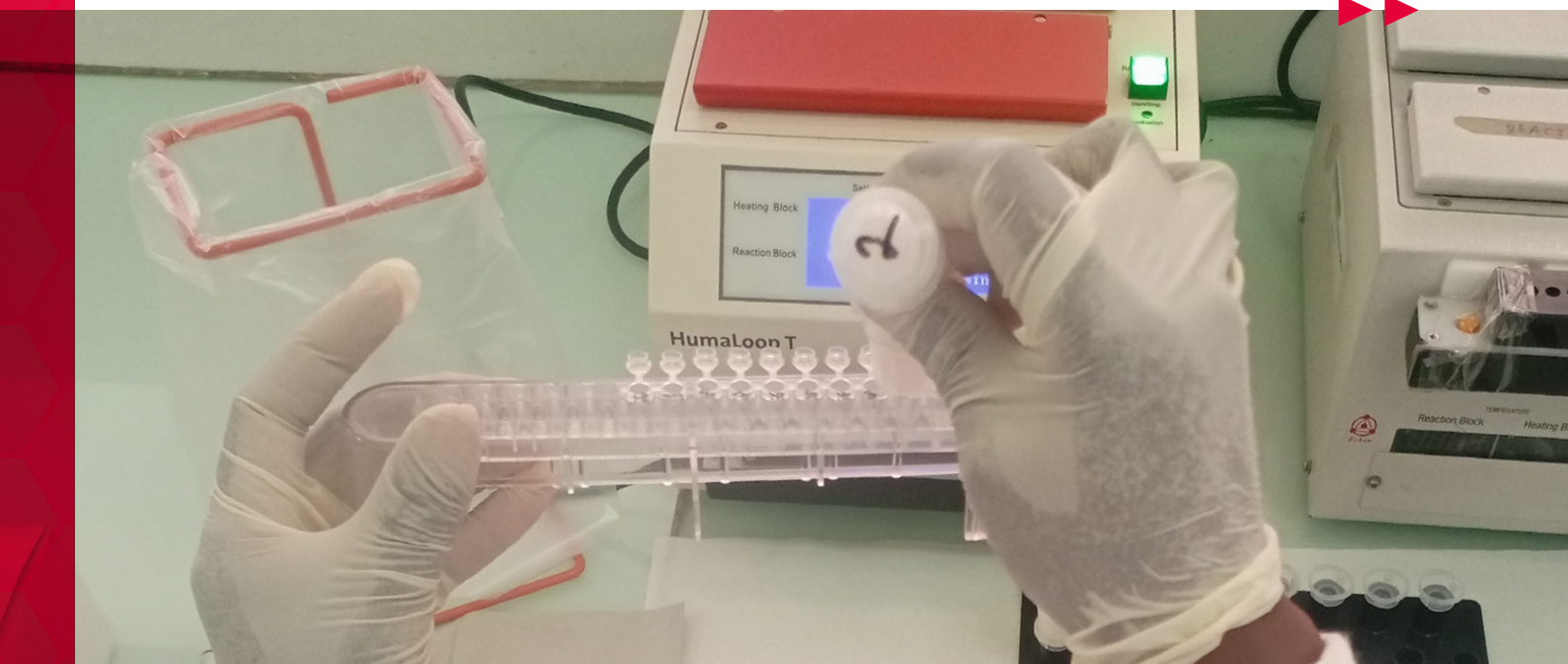
Based on the country experiences described in this document, TB-LAMP has two main use cases:

1. **Replacement of microscopy at relatively high capacity testing sites where Xpert is not available or cannot be used due to infrastructure constraints, with transport of only TB-LAMP positive specimens to Xpert or Truenat testing sites.**

   TB-LAMP requires simple instrumentation that is not affected by brief power outages and can be run at temperatures up to 40°C, making it suitable for use in areas that have limited infrastructure. The larger the batch size of TB-LAMP (up to 14 samples per batch), the lower the cost per sample tested.

2. **Initial rapid molecular test for use in active case finding activities where large volumes of specimens have been collected following digital X-ray/CAD as a screening tool. All TB-LAMP positive specimens should be tested by Xpert or Truenat for rifampicin resistance.**

   TB-LAMP has a higher throughput than both Xpert (4-module instruments) and Truenat (all workstations), and can also be cost-effective. In an active case finding study conducted in villages in Cameroon, using symptom screening followed by on-site TB-LAMP as the initial diagnostic test and subsequent Xpert for TB-LAMP positive samples, a 75% reduction in turnaround time and significant cost-savings were found in the second year of the campaign compared to a symptom screen and off-site Xpert strategy.[11]



▶▶ CONCLUSION ▶▶

To fully realize the WHO recommendation to use rapid molecular diagnostics for detecting TB, high TB burden countries need to make a shift from relying on sputum smear microscopy. To guide the selection and planning for implementation of rapid molecular tests, it is crucial to consider how the tests can complement each other and can be used in a manner depending on the available infrastructure and with organization of sample referral networks at the testing sites. TB-LAMP is particularly suitable for settings with limited infrastructure, where it can replace microscopy or serve as an initial bacteriological test during community-based active case finding campaigns. Overall, an integrated approach that combines various TB diagnostic tests can maximize the benefits of molecular testing and improve TB diagnosis in high TB burden countries.

▸▸ Acknowledgments

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Dr. Ramon Basilio
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IMAGES WERE PROVIDED BY HUMAN DIAGNOSTICS & DR VALERIE DONKENG OF THE CENTRE PASTEUR DU CAMEROUN

ANNEX 1

TB-LAMP INSTRUMENTS AND COMPONENTS PRICING

The required instruments, reagent kits and consumables are available in the Stop TB Partnership GDF Catalog. GDF-negotiated concession prices offered to the public sector in 145 high burden and developing countries.[17] The global distributor of TB-LAMP is HUMAN Diagnostics, Wiesbaden, Germany. The GDF diagnostic ordering list also includes a detailed description of what the installation, training and maintenance covers; however, travel and accommodation of HUMAN service providers is not included, and will be charged to the recipient by HUMAN.

Equipment

GDF Product Code	Description	Number of units/pack	Price (USD)
106632	HumaLoop T	1	$2,695.00
106663	HumaX ITA microcentrifuge	1	$632.50
106633	HumaTurb C + HumaTurb A	1C & 1A	$17,490.00
106636	HumaHeat	1	$1,056.00

Reagents

GDF Product Code	Description	Number of units/pack	Price (USD)
106634	Loopamp MTBC Detection Kit	2 x 48 tests	$219.00
106635	Loopamp PURE DNA Extraction Kit	90 tests	$275.00

Consumables

GDF Product Code	Description	Number of units/pack	Price (USD)
106643	Pipette-60 set for TB-LAMP	1	$42.00

Service & maintenance

GDF Product Code	Description	Number of units/pack	Price (USD)
106637	Installation	1	$1,424.50
106638	Training	1	$275.00
106639	Maintenance after year 1	1	$1,358.50
106640	Maintenance after year 1 plus 1-year warranty extension	1	$1,842.50

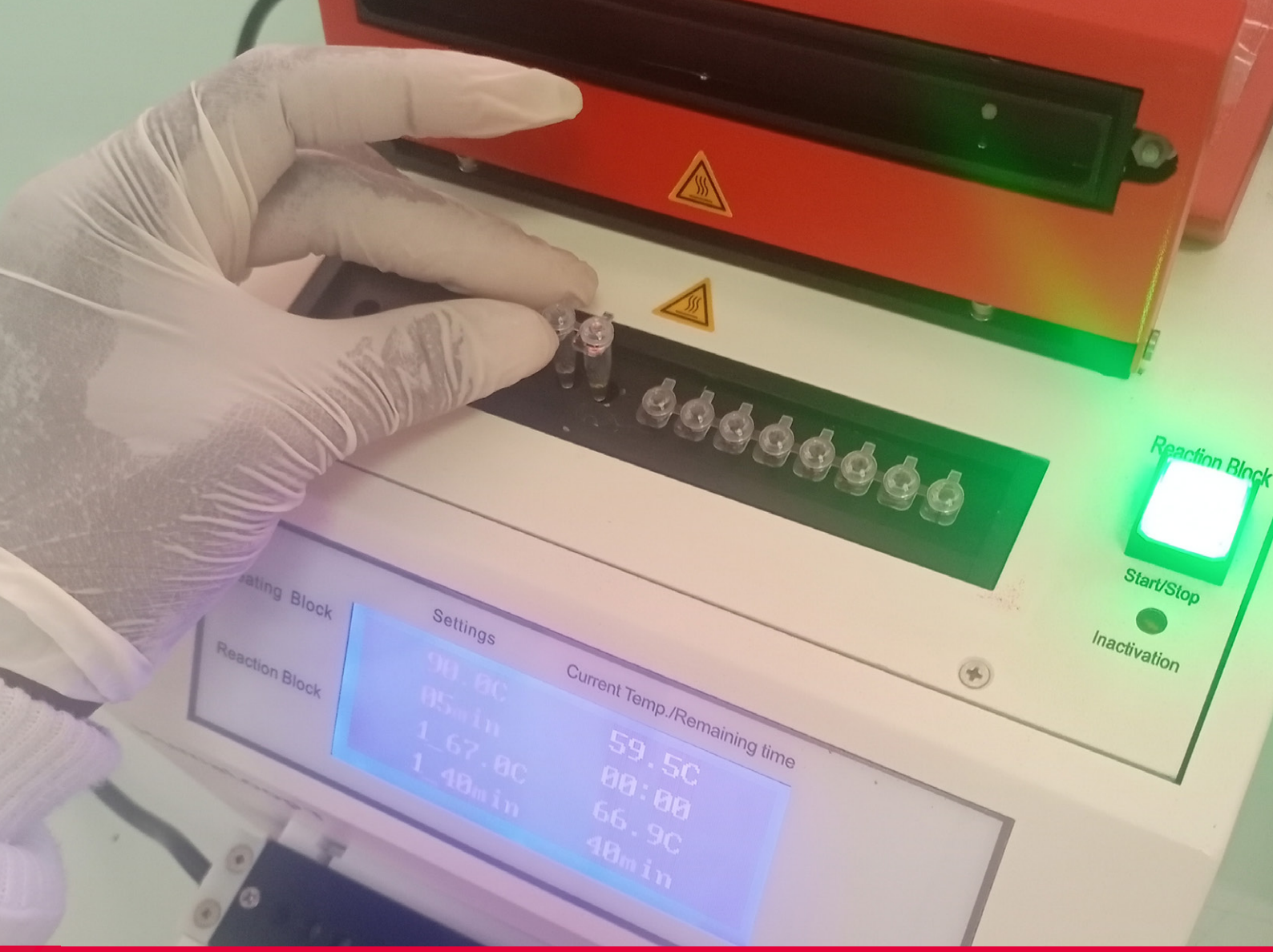
17. *Stop TB Partnership GDF Diagnostics, Medical Devices and Other Health Products Ordering List (2023).* https://www.stoptb.org/global-drug-facility-gdf/gdf-product-catalog

ANNEX 2

TB-LAMP COST PER SAMPLE TESTED UNDER VARYING BATCH SIZES

The table below shows how the cost per patient tested varies under different scenarios of batch efficiency. When batch sizes are maximized, i.e., 6 cycles testing 14 samples each, each extraction and detection kit can be used to test 84 samples. The cost per patient sample for reagents (extraction and detection tests) and consumables (Pipette-60 set) depends on the batch size of the cycle given the need for positive and negative controls in each cycle.

Number of patient samples in batch	Cost per patient sample (in USD)
14 (maximum efficiency)	$6.00
10 (70% efficiency)	$6.22
8 (57% efficiency)	$6.41
7 (50% efficiency)	$6.55
6 (43% efficiency)	$6.73
5 (35.7% efficiency)	$6.99
4 (28% efficiency)	$7.38
3 (21% efficiency)	$8.02
2 (14% efficiency)	$9.31
1 (no batching)	$13.17



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