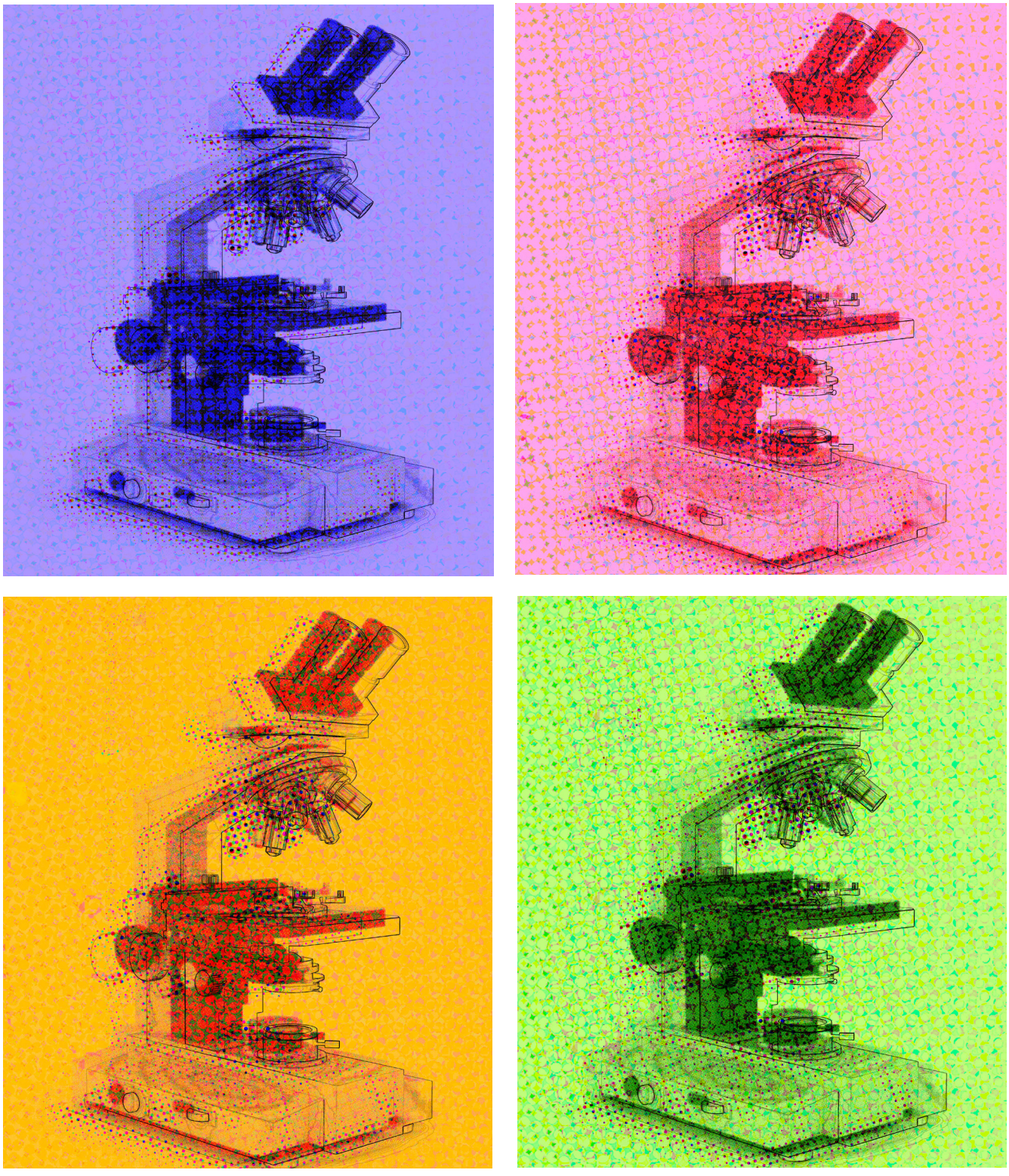
AN ACTIVIST'S GUIDE TO

Tuberculosis Diagnostic Tools



FEBRUARY 2017

TAG
Treatment Action Group

This guide was written by Khairunisa Suleiman and edited by Erica Lessem.
Thanks to Dr. Claudia Denkinger (FIND), Bryn Gay (Treatment Action Group), and Dr. Wayne van Gemert (World Health Organization), who generously contributed their time and expertise to reviewing this guide.
Layout by Hollander Snow Studio, Inc.

ABOUT TAG

Treatment Action Group (TAG) is an independent AIDS research and policy think-tank fighting for better treatment, a vaccine, and a cure for AIDS. TAG works to ensure that all people with HIV receive lifesaving treatment, care, and information. We are science-based treatment activists working to expand and accelerate vital research and effective community engagement with research and policy institutions. TAG catalyzes open collective action on the part of all affected communities, scientists, and policy makers to end AIDS.

ABOUT THE TB/HIV PROJECT

TAG's TB/HIV Project works to improve research, programs, and policy for people living with TB and HIV.

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ISBN: 978-0-9983966-1-3

Table of Contents

Introduction

Diagnosis refers to detecting a disease or condition. Understanding tuberculosis (TB) diagnosis is critical to understanding why the world is currently failing to end TB. To treat a person with TB infection or disease, one must first find and diagnose them. An estimated 41% of cases of active TB disease went undetected in 2015.[1] This means that an estimated 4.3 million people went without proper TB care in 2015, leaving them ill and at risk of death and with the potential to transmit disease to others.[2] Closing this massive gap will require much better use of the current diagnostic methods, as well as research into faster, simpler, more accurate, and less expensive options. Some challenges that need to be addressed through better access and research include:

Access Challenges

- lack of coordination among funders to ensure that resources are used efficiently;
- slow uptake of World Health Organization (WHO)-recommended tools as a result of inadequate budgeting and implementation in national programs;
- delays for diagnosing and treating TB, often caused by poor specimen transport (transport of bodily sample, such as sputum) and delayed return of results and inefficient linking person to medical care;
- little investment in laboratory capacity strengthening (improving the machines and infrastructure of the laboratory and the skills of laboratory workers);
- poor linkage to prevention or treatment, including especially poor access to children (who often do not test positive for TB even when disease is present), and to therapy to prevent active TB disease in high-risk groups such as children who live with someone with TB, and people with HIV;
- patients may not be able to afford diagnosis and treatment costs, as diagnosis and treatment are not always free in all public sector facilities, and there is limited reimbursement or free cost services in private sector;
- services provided by private sector health care providers not following national policies.

Access Needs

- adopt tools consistent with WHO recommendations, with adequate budgeting and implementation in national programs;
- absorb all unspent Global Fund to Fight AIDS, TB, and Malaria money that is allocated to TB programs;
- reduce delays for diagnosing and treating TB by ensuring adequate access to diagnostics near patient—or smooth specimen transport to where diagnostics are available—and quick return of results and efficient linkage to care;
- have all patients have access to drug-susceptibility testing (DST), at least for rifampin-resistance testing;
- increase investment in laboratory capacity strengthening.

Research Challenges

- little investment in basic science for identifying new markers of TB infection, disease, improvement, or worsening that could eventually be used in diagnostic tests (basic science for TB received just USD $139.8 million in 2015, out of a projected need of $455 million);
- insufficient investment in developing diagnostic tools (in 2015, only $62.8 million were invested out of a projected need of $364 million).[3]

Research Needs

- advocate for new, more sensitive, simpler, and cost-effective diagnostic tools;
- develop fast, non-invasive (do not need to be introduced into the body), and accurate tests to detect TB in children;
- develop a non-sputum-based test, as sputum (coughed up mucus) is challenging for many people (especially children and people with HIV) to produce, and cannot be used for finding TB outside the lungs.[4]

MORE LIMITATIONS OF CURRENT TB TESTS

More limitations of the current TB tests are listed below.

- There is no rapid test to detect TB in all populations (such as children or people with HIV or extrapulmonary TB) with first-line diagnostics
- Most TB tests require a good sputum specimen, which some patients are not able to produce
- There is no rapid test to detect resistance to drugs other than rifampin and isoniazid
- Some tests have low accuracy, either as a result of low sensitivity (high false negative results) or low specificity (high false positive results)[5]
- There is no point of care (POC) test that can be used in low-level health facilities, such as health clinics
- Most tests need electricity, equipment, and infrastructure[6] (for example, high biosafety levels and or lots of space or spare parts)
- Most are costly, so national governments often have to rely on donor support. There are also high add-on costs (such as importation and distribution costs) that make tests and testing unaffordable[7]
- Other than DNA, there are no good biomarkers (biological properties such as cells that give an indication of a disease or infection) that predict TB immunity, disease, or cure[8]

This guide describes the existing options for detecting TB infection and disease and offers priorities for advocacy for improving access to quality TB diagnosis as well as for accelerating research into better diagnostics—all with an eye towards guiding TB care towards ending TB. This guide organizes tests by whether they are used to detect active TB or TB infection. For active TB, the guide organizes tests according to a concept developed by Dr. Madhukar Pai, “see the bug, grow the bug, multiply the bug,” and also describes drug-susceptibility testing (DST) and how to diagnose TB in special populations such as children, people with HIV, and people with extrapulmonary TB (EPTB). For TB infection, the guide describes the available options for detection and their limitations. The guide will also note tests that are not recommended (though are unfortunately still used). Finally, the guide offers a comparison of all diagnostic methods (See Summary of TB diagnostic tests).

KEY DEFINITIONS AND ACRONYMS

CAD:	computer-aided detection, software that may support the automatic reading of chest X-rays to screen for tuberculosis
DNA:	deoxyribonucleic acid, a type of genetic material
DR-TB:	drug-resistant tuberculosis, meaning that the infecting strain of bacteria is not killed by a drug that is usually effective
DS-TB:	drug-sensitive tuberculosis, meaning the infecting strain of bacteria can still be killed by first-line drugs (rifampin, isoniazid, pyrazinamide, and ethambutol)
DST:	drug susceptibility testing (test to see whether drug will still work against the infecting strain of *Mycobacterium tuberculosis*)
EPTB:	Extrapulmonary tuberculosis (tuberculosis outside of the lungs)
HIV:	human immunodeficiency virus (a virus that weakens the immune system if untreated)
ICT:	information and communications technology
IGRA:	interferon gamma release assay (a test for tuberculosis infection described in this guide)
LAM:	lipoarabinomannan (a compound in the cell wall of *Mycobacterium tuberculosis* that is present in people with active tuberculosis disease)
LED:	light-emitting diode (an instrument that gives off visible light when an electric current passes through it)
LPA:	line probe assay (a test for tuberculosis and drug susceptibility testing that is described in this guide)
LTBI:	latent tuberculosis infection (infection with the *Mycobacterium tuberculosis* that is not causing active tuberculosis disease; LTBI does not make someone feel sick and is not transmitted to others)
MDR-TB:	multidrug-resistant tuberculosis (tuberculosis that is resistant to the two most powerful first-line drugs used to treat it, rifampin and isoniazid)
MTB:	*Mycobacterium tuberculosis* (the organism that causes tuberculosis infection and disease)
NAAT:	nucleic acid amplification test, a test that amplifies and detects genetic material; many tuberculosis tests explained in this guide detect tuberculosis by detecting the genetic material of *Mycobacterium tuberculosis*
POC:	point of care; in terms of tuberculosis diagnosis, a POC test is a test that is available at the lowest level of a health facility, such as a health clinic

KEY DEFINITIONS AND ACRONYMS

PPD:	purified protein derivative (a component of tuberculin skin testing, described in this guide)
QA:	quality assurance
RNA:	ribonucleic acid, a type of genetic material; NAAT can detect either DNA or RNA
RR-TB:	tuberculosis that is resistant to rifampin, one of the most powerful drugs that are commonly used to treat it
TB:	tuberculosis
TB CAB:	Global TB Community Advisory Board, an independent group of research-literate tuberculosis research and access activists
TB LAMP:	loop-mediated isothermal amplification (a technique used in a test with the same name for the detection of tuberculosis)
TST:	tuberculin skin test (a test for detecting the presence of MTB, which is described in this guide)
USD:	United States or American Dollars
WHO:	World Health Organization
XDR-TB:	extensively drug-resistant tuberculosis (multidrug-resistant tuberculosis that is also resistant to a fluoroquinolone [a type of second line drug] and a second-line injectable)

Diagnosing active TB disease

DETERMINING WHOM TO TEST

Not everyone needs to be tested for TB—unnecessary testing can needlessly expose people to risk and waste resources. There are some simple tools that can guide the decision about who should take a TB test. These tools are not very specific (that is, they cannot accurately determine whether a person has TB), but they are very sensitive (that is, they are unlikely to miss a case of TB), so they can help to 'rule out' active TB or determine who should receive microbiological TB testing (described in the next section). These can also be called triage or screening tools, as they help to determine who does or does not need further testing for TB. The two main screening tools are symptom screening and chest X-ray.

Symptom screen

Although TB shares symptoms with many other illnesses, most people with TB have some of the following symptoms:

1. current coughing (for any duration)[9]
2. night sweats
3. weight loss
4. fever
5. coughing up blood (called hemoptysis).[10]

People should go for screening when they have one or more of the above listed symptoms.

National and local TB programs should organize activities to regularly screen for symptoms among high-risk groups, and people with the above symptoms should be followed up for further evaluation. High-risk TB groups can be categorized by:

(1) Community (for example, areas with high TB prevalence such as slums, immigrants from settings with a high prevalence of TB, and people in close contact with someone with TB);

(2) Hospital departments and primary health care centers (for example, people previously treated with TB, people living with HIV and people attending HIV testing, undernourished people, people with diabetes, and other groups who have compromised immune systems);

(3) Type of residence (incarcerated people and detention center staff, people living in shelters, people living in immigration and detention centers, people in congregate settings, such as military);

(4) Workplaces (for example, health care workers, miners, and other people employed in workplaces with a high prevalence of TB).[11]

Activists should:

- conduct treatment literacy in their communities so that community members are aware of the symptoms of TB and can present themselves earlier to a health care facility;
- encourage their national and local TB programs to institute policies and practices for symptom screenings in high-risk groups.

SYMPTOM SCREEN | SCREENING TOOL | CANNOT DETECT DRUG RESISTANCE	
Advantages	**Disadvantages**
▪ Low cost ▪ Low technology ▪ Rapid ▪ Non-invasive ▪ No special risk to person being evaluated or to health care worker (if infection control measures are in place)	▪ May incorrectly indicate TB, as many health conditions can have the same symptoms (low specificity leading to false positive TB cases) ▪ May miss cases, as some people with active TB do not have typical TB symptoms in the early stages of the disease (imperfect sensitivity) ▪ Cannot be used to screen for TB outside of the lungs ▪ Needs a lot of resources in relation to the number of persons identified with TB if high-risk groups are not targeted (low yield)

Source: World Health Organization. Improving early detection of active TB through systematic screening. Geneva. World Health Organization; 2013. Available from http://www.who.int/tb/publications/tbscreening_factsheet.pdf?ua=1 (accessed 2016 August 9)

Chest X-ray

X-ray is a quick, common test that can create an image of the structures inside the body; as such, it is used for many different purposes. X-rays of the chest can help to identify TB in the lungs (known as pulmonary TB): air in the lungs normally shows up as black, whereas if there is damage to the lungs from TB (in the form of lesions), the X-ray will show abnormal grey or white shadows.[12] Digital chest X-ray is a more modern form of X-ray that, instead of using film and slides, produces digital images that can be read on a computer (including by experts not on site). The WHO recommends chest X-ray as an essential tool to end TB, noting its sensitivity for screening for active TB, its importance for diagnosing childhood TB, how it can improve the efficiency of using GeneXpert MTB/RIF (a test described in further detail later), its utility in assisting diagnosing TB in people with HIV, and its role in ruling out active TB before treating latent TB infection.[13]

Both conventional and digital X-rays can be used to screen for TB. However, given that an abnormal chest X-ray could also mean that there are other health issues, such as lung cancer or pneumonia, follow up tests are required. Chest X-rays cannot rule out TB outside of the lungs or TB in people with HIV (who can have chest X-rays that do not indicate TB, even when they have TB).

WHO guidance also notes the availability of computer aided detection (CAD), such as a software called CAD4TB, may help the people reading X-rays in the diagnosis of TB. The makers of this software suggest that it can automatically analyze X-rays to detect anything abnormal and indicate the likelihood of active TB, which is especially useful in settings without many skilled personnel.[14] The WHO has not recommended the use of computer-assisted reading tools, but will review the evidence and may make a recommendation in 2017.

Activists should:

- advocate for the availability of no-cost digital chest X-ray in their communities to aid in the diagnosis of TB and other diseases, and to reduce the financial burden on patients;
- advocate for health services to provide good referral to follow-up tests in addition to chest X-ray to confirm the presence of TB or other health conditions;
- monitor upcoming WHO review of evidence about computer-assisted reading tools (for example, CAD4TB), to see whether these tools may help to make the use of chest X-ray more efficient and accessible.

CHEST X-RAY | SCREENING TOOL | CANNOT DETECT DRUG RESISTANCE

Advantages	Disadvantages
▪ Inexpensive ▪ Fast ▪ Widely available in urban areas ▪ Highly sensitive, so can rule out active pulmonary TB (a negative result means no TB)	▪ Must be followed up with additional testing to confirm TB (low specificity) ▪ Can only be used to detect TB in lungs ▪ Chest X-ray is a screening test as opposed to a diagnostic test ▪ 10%–15% of culture-positive TB cases are missed by chest X-ray (imperfect sensitivity); in people with HIV, as many as 30% of patients with TB might not be detected by chest X-ray ▪ Not all lesions may be attributed to TB (low specificity) ▪ Cannot tell whether an individual has drug-susceptible or drug-resistant TB ▪ Special equipment with adequate input power required ▪ Trained personnel for operation and interpretation required ▪ Health care workers can interpret the same X-ray differently (inter- and intra-reader variability) ▪ Limited availability in low- and middle-income countries, especially in rural settings ▪ There is no universal and agreed upon reporting system ▪ Exposes person to small amounts of radiation, requiring special procedures and equipment (such as wearing protective clothing) for person screened and the health care worker
Additional advantages of digital chest X-ray include: ▪ Few consumables, such as film, required (therefore lower cost per test and less chance of shortages) ▪ Results available immediately ▪ Lower radiation dose ▪ More convenient portable systems allow for use in mobile health units and outreach activities ▪ Allows for sending images electronically to X-ray technicians not on site, which can improve quality and allow for research ▪ Easier to store and find images ▪ Better image quality	

Sources: CheckTB. Innovative chest X-ray solutions supporting TB prevalence studies. Geneva. Stop TB Partnership; 2009. Available from: http://www.who.int/tb/advisory_bodies/impact_measurement_taskforce/meetings/prevalence_survey/chest_x_ray_solutions.pdf (Accessed 2016 August 8)

World Health Organization. Chest Radiography in Tuberculosis Detection. Geneva: World Health Organization; 2016. Available from: http://www.who.int/tb/publications/Radiography_TB_factsheet.pdf (Accessed 2017 January 30)

MICROBIOLOGICAL CONFIRMATION

Ideally, TB should be diagnosed through what is called microbiological confirmation; that is, detecting the bug that causes TB (the bacterium *Mycobacterium tuberculosis*, MTB). Identifying the presence of the bug itself provides a certain diagnosis and is also necessary for determining which drugs the strain of the bug is susceptible or resistant to in order to guide treatment. There are several different tests that can do this, and we group them here according to a concept developed by TB diagnostics expert Dr. Madhukar Pai: see the bugs, grow the bugs, multiply the bugs.

With the exception of LAM testing (which is used for a specific patient population; see below), microbiological tests for TB are not POC. This means that many sites where patients access care do not have all of the required tests in the facility. Thus, an efficient specimen transport and referral system is critical for a TB laboratory network. Settings without such systems do not provide patient access to the needed high-quality and rapid TB detection and DST, or patients may be expected to travel to other facilities, which may incur significant costs to the patient and delay diagnosis.

Activists should:

- advocate for strong specimen transport and referral systems, allowing for rapid and reliable return of results and reducing the financial burden on patients.

See the bug —Sputum smear microscopy

The oldest way to detect TB is by looking under a microscope for the presence of the TB bug in the sputum (coughed up phlegm) of people thought to be sick with TB. Because the TB bug has a fatty cell wall, it can be stained using acid-containing liquids to make it visible under a microscope. This is a relatively simple and fast way to diagnose TB, and it remains the most common method used around the world. Microscopy is also important in treatment monitoring—if the person's sample remains sputum smear positive after two months of TB treatment, it is a sign that treatment is not working. However, sputum smear microscopy has many limitations. By definition, it relies on sputum, which is difficult for people (especially children) to produce and is the wrong sample for diagnosing TB outside of the lungs. Other bugs in the *Mycobacteria* group called non-tuberculosis mycobacteria also show up this way (imperfect specificity), and patients may be wrongly treated for TB when they have another mycobacterial disease. Microscopy also misses many cases of TB (low sensitivity), especially among people living with HIV and among children. Fluorescence, which is a technique that makes substances appear to glow, can help to improve the sensitivity of microscopy by making the MTB bug easier to visually detect. In 2010, the WHO endorsed a further improved method of smear microscopy called LED fluorescence microscopy, which increases the sensitivity of smear microscopy by using a high-powered light to help guide detection of the bug. The WHO recommends that LED microscopy should, in stages, replace normal light microscopy.[15,16]

Activists should:

- advocate for the use of more accurate tests (high sensitivity and specificity) such as GeneXpert (see below) as the initial test for diagnosing TB;
- when smear is used, call for the replacement of conventional light microscopy with LED microscopy.

SPUTUM SMEAR MICROSCOPY | ACTIVE TB DIAGNOSIS | CANNOT DETECT DRUG RESISTANCE	
Advantages	**Disadvantages**
▪ Simple, inexpensive, and rapid ▪ Suitable for low-level and high-level laboratories, including labs with low biosafety levels ▪ Can be used for treatment monitoring ▪ Widely available	▪ Relies on sputum ▪ Can only be used for pulmonary TB ▪ Low sensitivity, especially for extrapulmonary TB, people with HIV and in children ▪ Cannot differentiate between MTB and other types of bacteria from the same group ▪ Cannot differentiate drug-susceptible from drug-resistant TB ▪ It is difficult to implement good quality assurance (QA), which is vital to ensure high-quality results
Additional advantages of LED microscopy include: ▪ Higher sensitivity (by 10%) than normal light microscopy ▪ Inexpensive light source with long battery life ▪ Requires less power than normal/conventional fluorescence microscopy ▪ Reduces time to detect TB compared to conventional microscopy	

Source: Implementing TB diagnostics. World Health Organization. 2015 Geneva: WHO policy framework.
Available from http://apps.who.int/iris/bitstream/10665/162712/1/9789241508612_eng.pdf?ua=1 (Accessed 2016 July 28)

Grow the bug— Culture

The most accurate way to diagnose TB—the gold standard—is through culture. Culture means taking sputum or another sample from the body that is thought to contain the TB bug, decontaminating it (to stop the growth of other non-TB organisms), and giving it time to grow on a specific material or medium. The medium can be solid or liquid. If there are TB bugs in the sample, their growth can either be seen by the human eye or detected automatically by a machine. Culture can also detect drug-resistant TB (DR-TB) [see box on page 11]. Culture should be used for monitoring treatment for multidrug-resistant TB (MDR-TB); the WHO recommends taking and culturing samples monthly.[17]

There are two types of culture, liquid and solid. Both solid and liquid culture must take place at central reference laboratories. Liquid culture can be done automatically and is much faster (about two weeks) than solid culture (which takes up to two months). Liquid culture can be done on Becton Dickinson's BACTEC MGIT automated machine, which costs $38,950 and $16.88 per sample.[18,19]

Culture results are often received too late to inform treatment decisions or get lost along the way. For culture to have the effect it could on outcomes, its implementation requires good systems to ensure rapid turn-around time (sample transport solutions) and delivery of information (information and communications technology or ICT solutions).

Activists should:

- advocate for liquid culture capacity at reference laboratories in their countries, including sufficient capacity for treatment monitoring (liquid or solid culture) to know whether patients are responding to DR-TB treatment;
- advocate for efficient specimen transport and information networks so that clinicians can refer specimens to reference laboratories and get results back in as fast a manner as possible;
- monitor machine and reagent prices[i] and ensure Becton Dickinson makes concessional (reduced) pricing available for all low and middle-income countries.

CULTURE | ACTIVE TB DIAGNOSIS | CAN DETECT DRUG RESISTANCE	
Advantages	**Disadvantages**
▪ Gives definite diagnosis of TB ▪ Can detect 30%–50% more cases than microscopy (high sensitivity) ▪ Provides the needed isolates for drug susceptibility testing (DST) ▪ Necessary for monitoring DR-TB treatment	**Disadvantages of solid culture include:** ▪ Results take a long time (about two months) due to slow growth ▪ High biosafety level requirements ▪ Highly trained staff needed ▪ Electricity needed*
Additional advantages of liquid culture include: ▪ can process higher number of samples than solid culture (10% higher) ▪ Faster than solid culture due to automation	**Disadvantages of liquid culture include:** ▪ Prone to contamination; rapid specimen transport is critical ▪ More expensive than solid culture; the manufacturer is not making concessional prices available to all countries that need them ▪ Need for reagents to run a test* ▪ Higher biosafety requirements needed due to large volumes of infectious samples

Source: Implementing TB diagnostics. World Health Organization. 2015 Geneva: WHO policy framework. Available from http://apps.who.int/iris/bitstream/10665/162712/1/9789241508612_eng.pdf?ua=1 (Accessed 2016 July 28)

*both for solid and liquid culture

i. FIND negotiated pricing can be found on http://www.finddx.org/find-negotiated-product-pricing/

Drug-susceptibility testing (DST)

DST is very important for guiding TB treatment as it tells the clinician whether a strain of TB is or is not susceptible to a certain drug; in other words, whether that drug will work or not. There are two types of DST methods: phenotypic and genotypic. The phenotypic method uses culture. The bacteria colonies that grow are placed on plates that contain the drug in question. If the bacteria grow, it means that they are resistant to the drug; if they do not grow, they are susceptible to it. Phenotypic DST can use liquid or solid culture and can be done through direct or indirect testing. Direct testing is when the TB sample is directly placed on the drug-containing media; indirect testing is when a pure MTB culture grown from an original sample is used.[20] Genotypic methods are described in more detail in the section below. Overall, phenotypic DST takes longer to provide results than genotypic DST.

Multiply the bug — Nucleic acid amplification

Nucleic acid amplification testing (NAAT) is a method used to detect TB as well as other pathogens. NAAT tests look for the bug's genetic material or molecules (known as DNA or RNA) in samples, and are therefore called genotypic or molecular testing. Because resistance to drugs is written into the MTB bug's genetic information, many NAAT tests can also do genotypic DST. NAAT is much faster than culture because this technology multiplies the genetic material of the bug to more easily detect it, rather than waiting for the bug to grow. Another advantage is that many samples can be processed at once (called a high throughput).[21] Molecular methods are also less likely to be affected by contamination. There are currently three different kinds of WHO-recommended NAAT testing.

GeneXpert MTB/RIF

Cepheid's GeneXpert system is a cartridge-based NAAT that can be used for many different indications. The WHO-endorsed MTB/RIF cartridge can simultaneously detect TB and resistance to rifampin, and can run from 1 to 80 samples at a time (depending on the instrument that is bought).[22] A fully automated and rapid test, GeneXpert MTB/RIF works in about two hours. It performs very well on sputum, and its sensitivity is better than microscopy, including for people with HIV. It can also be used in other samples to diagnose EPTB (see page 16). A four-cartridge GeneXpert machine at a negotiated price for eligible countries is about USD 17,000 and one cartridge costs about USD 10.

Cepheid is working on two other cartridges: Ultra, which should have improved sensitivity and improved detection of resistance to rifampin, and an XDR cartridge that can detect resistance to isoniazid and second-line drugs, and therefore better guide the rapid starting of the most effective treatment for a given strain of TB once rifampin resistance has been identified.

Responding to some of the challenges around GeneXpert's introduction, Cepheid is also developing a new platform called Omni. The original GeneXpert platform has a problematic reliance on electricity, and a tendency to produce more and more errors over time when exposed to dust. Cepheid's Omni, a single-cartridge platform, is supposedly more rugged and can use battery or solar power and can withstand dust and heat, and is expected to have fewer training requirements. Anticipated timelines are to release Omni by Q3 2017.

At the time of writing, Cepheid is about to be acquired by a larger diagnostics company called Danaher, and states that development of these products will continue. Cepheid says outside funding is needed to expedite the

XDR cartridge development. Tests similar to GeneXpert are being developed by competitors, such as Molbio's TrueNAT, but have not yet received WHO approval.

Activists should:

- advocate for the use of GeneXpert as the first test for TB for all patients;
- advocate for efficient specimen transport networks so that clinicians can refer specimens to sites with GeneXperts and get results back in as fast a manner as possible;
- continue to call for reduced, volume-based pricing of Xpert machines and consumables (for example, cartridges), so that low- and middle-income countries do not rely on donors to buy and service Xpert instruments;
- call on Cepheid and Danaher, which (at the time of writing this guide) is about to acquire Cepheid, to prioritize and expedite research and development of the Omni platform, and the Ultra and XDR cartridges;
- call on donors to support development and trialing of alternative tests in the pipeline such as Molbio's TrueNAT.

GENEXPERT MTB/RIF | ACTIVE TB DIAGNOSIS | CAN DETECT RIFAMPIN RESISTANCE	
Advantages	**Disadvantages**
▪ Can detect TB and rifampin resistance at the same time ▪ Rapid (results in in less than two hours) ▪ High sensitivity (88%) and high specificity (99%) when compared to liquid culture in sputum samples ▪ Low biosafety level requirements (compared to culture) and similar to microscopy ▪ Minimal training of personnel ▪ Can detect both pulmonary TB and EPTB ▪ Same system can be used for other conditions such as early infant HIV diagnosis and viral load monitoring (pending WHO approval)	▪ Requires stable uninterruptable electricity (not suitable for regions with power cuts) ▪ Operating temperature should not exceed 30 degrees Celsius and cartridge must be stored at less than 28 degree Celsius (in an air-conditioned room) ▪ Cartridges' shelf life must be monitored or are prone to being wasted (MTB/RIF's shelf life is currently 22 months; Ultra, if approved, will likely have a shorter shelf life) ▪ Security measures must be put in place to avoid theft of laptop or desktop computer ▪ Cannot be used to monitor TB treatment ▪ The instrument's accuracy needs to be checked (calibrated) every year

Source: Implementing TB diagnostics. World Health Organization. 2015 Geneva: WHO policy framework.
Available from: http://apps.who.int/iris/bitstream/10665/162712/1/9789241508612_eng.pdf?ua=1 (Accessed 2016 July 28)

Line probe assay (LPA)

Another type of NAAT test is the line probe assay (LPA). This kind of technology takes genetic material (DNA) from either clinical specimens or MTB isolates (colonies of MTB that have been separated from all the other stuff in sputum or another sample using culture). Then the part of the genetic material that codes for resistance to a certain drug is multiplied, and it attaches to specific colored probes. These colors appear on a paper strip, allowing the test results to be read visually by seeing the resulting color. Depending on whether the LPA can detect resistance to first- or second-line drugs (isoniazid and rifampin, or some fluoroquinolones and injectables, respectively), it is called first-line or second-line LPA. First-line LPA can detect resistance to rifampin and isoniazid in less than two hours. WHO endorsed first-line LPA in 2008, before GeneXpert was endorsed in 2010.[23] WHO-endorsed first-line LPA is produced by two different companies, Hain's Lifescience and Nipro. FIND-negotiated pricing per test for first-line LPA is about USD 7.95 and each kit (96 tests) costs USD 795.[24] The estimated cost of all of the parts of the machine to run a test amounts to an expensive USD 46,580.[25]

Second-line LPA, called MTBDRsl, received WHO endorsement in 2016 and is manufactured by Hain Lifesciences.[26] MTBDRsl can detect resistance to some fluoroquinolones (ofloxacin and levofloxacin) and all second-line injectables (kanamycin, amikacin, and capreomycin), and ethambutol.[27] Given that it produces results quickly, it is very important for guiding who can take the shortened regimen. However, as it is imperfect at detecting resistance to other fluoroquinolones, such as moxifloxacin or gatifloxacin, their inclusion in a MDR-TB regimen is best guided by culture results.[28]

Activists should:

- advocate for the availability and use of second-line LPA in central reference laboratories to help allow appropriate treatment selection by a medical team;
- advocate for Hain to implement transparent, fair volume-based pricing for its LPA products to motivate uptake while working towards price reductions.

LINE PROBE ASSAY (LPA) | ACTIVE TB DIAGNOSIS | CAN DETECT DRUG RESISTANCE	
Advantages	**Disadvantages**
First-line LPA ▪ Can detect both rifampin and isoniazid resistance at the same time ▪ Rapid detection* (48 hours) of RR-TB and MDR-TB ▪ High throughput—can process up to 48 samples at the same time*	▪ Still requires culture and DST* ▪ Can only be used in central or reference labs or well-equipped regional labs. Lots of space is required (three separate rooms)* ▪ Cannot be used to guide choice of second-line drugs in individualized MDR-TB drug regimens ▪ Sensitivity to detect resistance to isoniazid is lower (about 85%) compared with culture methods ▪ Training of personnel is intensive*

LINE PROBE ASSAY (LPA) | ACTIVE TB DIAGNOSIS | CAN DETECT DRUG RESISTANCE (CONT'D)	
Advantages	**Disadvantages**
Second-line LPA (MTBDRsl) ▪ Once a patient is diagnosed as RR-TB or MDR-TB, second-line LPA can be used instead of phenotypic DST to detect fluoroquinolones and second-line injectables only ▪ For confirmed RR-TB or MDR-TB, direct sputum testing can be used, unlike first-line LPA. This shortens the time to treatment, as it is faster than first-line LPA ▪ Can detect second-line resistance to fluoroquinolones and second-line injectable TB drugs in a single working day (about 24 hours) ▪ Can detect both pulmonary and extra-pulmonary TB	▪ Cannot replace phenotypic culture-based DST for other second line drugs such as cycloserine or linezolid ▪ Only confirmed RR-TB and MDR-TB samples can be used for testing ▪ Normal phenotypic culture-based DST should be used in the follow-up evaluation of patients with a negative result especially in settings with a high pre-test probability for resistance to fluoroquinolones

Source: World Health Organization. The use of molecular line probe assays for the detection of resistance to second line tuberculosis drugs. Policy Guidance. Geneva. World Health Organization; 2016. Available from http://www.who.int/tb/WHOPolicyStatementSLLPA.pdf (Accessed 2016 July 28)

* same for second-line LPA

Loop-mediated isothermal amplification (LAMP)

TB loop-mediated isothermal amplification (LAMP) is a manual NAAT. With LAMP, DNA amplification and detection of a gene can be completed in a single step.[29] The test does not require sophisticated instruments and can be used at the peripheral health center level (closer to where people seek care, rather than a central laboratory), given biosafety requirements similar to sputum smear microscopy. The manufacturer of TB-LAMP is Eiken Chemical Company Ltd (Tokyo, Japan).

The advantages include the fact that TB LAMP is fast (it takes about 40 minutes) and it generates a result that can be detected with the naked eye under ultraviolet light. In August 2016, the WHO recommended that LAMP may be used for the detection of TB as a replacement test for smear microscopy for the diagnosis of pulmonary TB in adults with signs and symptoms of TB.[30] TB LAMP is a more sensitive test than smear microscopy, and 40% more patients are detected in smear-negative samples if TB LAMP is used as an add-on test once smear microscopy has been carried out. The WHO did not make a decision for or against the use of TB LAMP in the detection of TB in people with HIV due to the lack of evidence available.[31] TB LAMP cannot replace smear microscopy for treatment monitoring. TB LAMP does require electricity and temperature control.

Given the superior sensitivity and specificity and the ability to detect rifampin resistance up front by Cepheid's Xpert MTB/RIF test, and the anticipated POC platform Omni on the horizon that will have fewer training requirements, introducing TB LAMP does not appear to offer many benefits. Introducing a new test requires trainings, sorting out QA schemes and procurement, service and maintenance, etc. This is costly and time consuming, especially for settings with limited resources. LAMP equipment (about USD 730) is less expensive than GeneXpert, but the cost of Omni will be similar to the cost of the instruments necessary for TB LAMP, whereas the cost per test for TB LAMP is about the same as an MTB/RIF cartridge (EUR 7 or USD 9.7 per TB LAMP test, versus MTB/RIF at USD 10), which is not cost saving, especially considering that LAMP cannot detect

rifampin resistance. Each run of six LAMP samples requires running a positive and negative sample (therefore adding the cost of two additional tests per run). Thus, it is not really worthwhile to invest in the introduction of TB LAMP.

TB LAMP | ACTIVE TB DIAGNOSIS | CANNOT DETECT DRUG RESISTANCE	
Advantages	**Disadvantages**
▪ Fast—takes less than an hour to get results ▪ Low biosafety level, which is similar to microscopy ▪ Higher sensitivity than smear microscopy (by 15% more patients) ▪ Equipment more affordable than GeneXpert	▪ Cannot distinguish DR-TB from DS-TB ▪ Less specificity than smear microscopy—hence more false positive results (more people identified as having TB when they don't have TB) ▪ Worse than GeneXpert in terms of sensitivity ▪ Similar to GeneXpert, it CANNOT be used for treatment monitoring because it cannot distinguish between live and dead bacteria. Thus, sputum smear microscopy is still needed for treatment monitoring ▪ TB-LAMP requires several manual steps (around 10 steps) to perform the test and some of the steps are time and volume sensitive. Thus, it requires investments in terms of training and QA

Source: World Health Organization. The use of loop mediated isothermal amplification for the diagnosis of TB. Policy Guidance. Geneva: World Health Organization; 2016. Available from http://www.who.int/tb/features_archive/TB_LAMP/en/ (Accessed 2016 August 15)

Global Laboratory Initiative. Practical Considerations for Implementation of TB-LAMP. Geneva: Stop TB Partnership; 2016. Available from: http://www.stoptb.org/wg/gli/assets/documents/ (Accessed 2017 January 30)

Activists should:

- ensure national TB programs are not implementing LAMP in settings with high rates of MDR-TB or HIV, or in any setting that is already effectively using GeneXpert.

DIAGNOSING TB IN SPECIAL POPULATIONS

Extrapulmonary TB (EPTB)

EPTB is TB outside of the lungs. Globally, 17%–52% of people have EPTB.[32] When it is thought that someone might have EPTB, a sample must be obtained from the area thought to be infected with TB—for example, in lymph nodes, a liquid sample must be removed from the center of the lymph node by a needle.[33] Smear testing is highly likely to be negative, as the area of disease is not in the lungs. A combination of methods is used to test for EPTB, including NAAT and culture. GeneXpert is endorsed for EPTB diagnosis in selected samples, including from the lymph node, cerebrospinal fluid, and tissues.[34,35] GeneXpert is faster than culture, which is another option for testing samples from these sites in the body. Biopsy (examination of tissue from the body to detect the presence of a disease) sample testing at the presumed site of infection is also very useful. Empiric treatment should be used if the test results are negative, but there is still high clinical suspicion, and should be reviewed within one week of treatment to test whether the patient is responding to treatment.[36] Blood is not a sample for EPTB, and blood tests cannot be used to test for presumed EPTB.

Children

Children are much more likely than adults to progress to active disease if infected with TB, but diagnosing TB in children is difficult. GeneXpert is a more sensitive tool than sputum smear microscopy in the diagnosis of TB in children, and is the preferred method. However, even with GeneXpert, it can be difficult to accurately diagnose TB in children. This is because:

- it is difficult to get a sputum sample from children. Sometimes gastric lavage must be performed to get samples from children, which is invasive and requires specialized skills;
- the amount of bacteria in children is less than in adults (this is also called paucibacillary TB disease).

Thus, physicians often treat TB without microbiological confirmation; this is also called empirical treatment. Doctors can decide to start empirical treatment using the following methods:

- signs and symptoms of active TB (symptom screening);
- chest X-ray with images suggestive of TB disease;
- contact tracing (looking for the contacts of TB infected people such as house mates);
- history of a person who has infectious TB disease.[37]

People with HIV

People with HIV have a higher risk of developing TB (12–20-fold higher) than people without HIV.[38] People with HIV also have an increased risk of developing EPTB (40%–80% of HIV-positive people with TB have extrapulmonary disease, as compared with 10%–20% of HIV-negative people with TB).[39] People with HIV have fewer bacteria in their bodies (paucibacillary disease). Sputum smear microscopy therefore misses most cases of TB in people with HIV. As described above, chest X-rays are usually not a good way to screen for TB in people with HIV. GeneXpert is the recommended initial test for diagnosing TB in people with HIV.

LAM: for people living with HIV

Another important option for diagnosing TB in some people with HIV is LAM testing. Lipoarabinomannan (LAM) is an antigen in the cell walls of MTB. LAM is present only in people with active TB disease, and more so in people with immunosuppression (low immunity, such as from HIV). Alere produces the Determine LAM TB test, a lateral flow test that looks for LAM in urine (the test looks much like a typical urine-based pregnancy test). The WHO endorsed this LAM test in 2015, recommending it for use only in people with HIV with a CD4 cell count of less than or equal to 100/millimeter^3 or who are seriously ill. The test has low sensitivity (about 60%) in this population, and even lower sensitivity in other populations, so it should not be used in anyone other than the recommended population. Given the limited sensitivity of LAM testing, a negative test cannot be used to rule out TB and should be followed up with other testing.

Without the LAM test, it is very difficult to diagnose TB in people with HIV with low CD4 cell counts, and they are at extreme risk of dying from TB before getting a diagnosis. Many of these patients also have a hard time producing sputum, so this urine-based test can be very useful. Patients get their results in only 25 minutes,[40] it is a simple, non-invasive test, and costs about USD 2 per test. LAM is currently the only truly POC test for diagnosing TB, although it can only work in a small section of the population. LAM is also the only test ever shown to help reduce mortality (deaths) from TB in a clinical trial.[41] As such, it is a very important test to use in people with HIV with low CD4 cell counts. Unfortunately, use of LAM testing has been very low despite its life-saving potential in a very vulnerable population.

LAM | ACTIVE TB IN PEOPLE WITH HIV WITH LOW CD4 COUNTS | CANNOT DETECT DRUG RESISTANCE	
Advantages	**Disadvantages**
▪ Sample is easy to obtain: urine is easier to collect than sputum (especially in very sick patients) ▪ Fast—rapid dip stick test takes 25 minutes to get results ▪ Simple ▪ Inexpensive ▪ Assists in detecting TB in PLHIV with low CD4 cell counts or who are seriously ill	▪ Low sensitivity (56%) in people living with HIV who are seriously ill with TB symptoms, and suboptimal specificity. Both positive and negative results should be followed up with GeneXpert or other laboratory tests. ▪ Cannot tell about drug susceptibility ▪ Can only be used in a specific population with low CD4 cell count and who are seriously ill (low specificity in HIV-negative populations) ▪ Cannot differentiate MTB from non-mycobacteria ▪ A negative result should be followed up with another kind of test to rule out TB

Source: World Health Organization. The use of lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis and screening of active tuberculosis in people living with HIV. Geneva: World Health Organization; 2015. Available from: http://www.who.int/tb/areas-of-work/laboratory/policy_statement_lam_web.pdf (Accessed 2016 July 27)

Global Laboratory Initiative. Study finds LF-LAM-guided TB treatment initiation reduces mortality in HIV-positive hospital inpatients. Geneva: Stop TB Partnership; 2016. Available from: http://stoptb.org/wg/ gli/assets/documents/LF_LAM%20info%20note.pdf (Accessed 30 January 2017)

Activists living in settings with high burdens of TB/HIV should:

- urge their national TB and HIV programs to procure and roll out the LAM test immediately in the recommended population.

DIAGNOSING LATENT TB INFECTION

Treating latent TB infection (LTBI, or TB that is dormant and as such not currently making someone ill or capable of transmitting TB), or preventive therapy, is an essential part of preventing TB and helping to reduce the TB burden in a community or country. There are several options for diagnosing latent TB infection, which are described in this section. However, there is no gold standard for LTBI testing, the LTBI tests cannot distinguish active TB from latent TB, and there is no way of determining which individuals with latent TB infection will develop disease with the current tests. Given the huge benefit of preventive therapy in people with HIV and in children, and the challenges of testing for LTBI, WHO recommends starting people with HIV, and children under age five who live with people with TB, on preventive therapy without LTBI testing results,[42] as long as active disease can be ruled out. Indeed, the most important step to guiding preventive therapy is excluding active TB disease, as starting preventive therapy when someone actually has active TB disease can result in inadequate treatment, development of drug resistance, and passing the disease to someone else.[43]

There are tests available to test for LTBI in settings that do so. Tests for LTBI indirectly detect latent TB by detecting the human body's immune cell response, rather than looking for MTB directly. A positive test means that an individual has been exposed to MTB antigens, a part of the MTB cell that triggers an immune response in the human body, such as the creation of antibodies or memory T cells. Given that both tests measure the body's immune response, rather than identify MTB itself, there is no way of telling from a positive test whether someone is infected with a drug-resistant or drug-susceptible strain of TB.

Tuberculin skin test (TST)

Tuberculin skin test (TST) is the oldest method for detecting latent TB infection. TST is also referred to as the Mantoux test. The procedure involves injecting the arm with a tuberculin purified protein derivative (PPD). If there is TB infection, a bump will form (which should be measured in millimeters). The test results should be read after 48–72 hours.[44]

TST | LATENT TB DIAGNOSIS | CANNOT TELL IF ONE WILL DEVELOP TB IN THE FUTURE	
Advantages	**Disadvantages**
▪ Widely used and inexpensive ▪ Does not require special lab supplies or infrastructure	▪ Requires two patient visits ▪ Results are available in 48–72 hours ▪ Requires injection into the skin ▪ Adequately trained staff are needed ▪ Poor specificity for people vaccinated by bacille Calmette-Guerin (BCG), causing false positive results in the first ten years of vaccination** ▪ Poor sensitivity in immunocompromised individuals (for example, people with HIV) yields false negative results ▪ Test needs to be refrigerated

**Farhat M, Greenway C, et al. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? Int J Tuberc Lung Dis. 2006 Nov 1;10(11): 1192-204. Available from http://www.ingentaconnect.com/content/iuatld/ijtld/2006/00000010/00000011/art00003

Interferon gamma release assay (IGRA)

Interferon gamma release assays (IGRAs) use a blood sample to measure a person's immune reactivity to MTB. White blood cells from people with MTB infection release a small protein called interferon gamma when they meet MTB antigens. IGRA is performed by mixing fresh blood samples with antigens and controls to compare reactions.[45] The results are based on the amount of interferon gamma released.[46] There are two types of IGRAs recommended to detect TB infection: Qiagen's Quantiferon Gold and the T-SPOT.TB ELISPOT assay. These tests are faster (results within 24 hours) than TST and do not yield false positive results from previous BCG vaccination, unlike TST. The reason is that IGRAs use different antigens for stimulation than TST. As mentioned above, TST uses purified protein derivative, a mixture of more than 200 different compounds from different mycobacterial cells. In contrast, IGRAs use antigens that are relatively specific to MTB. As such, IGRAs have higher sensitivity and specificity than TST.[47]

IGRA | LATENT TB DIAGNOSIS | CANNOT TELL IF ONE WILL DEVELOP TB IN THE FUTURE	
Advantages	**Disadvantages**
▪ Requires one patient visit ▪ Faster than TST, results available in 24–48 hours ▪ High specificity in BCG vaccinated populations, do not cause false positive results, unlike TST	▪ Expensive ▪ Requires blood to be drawn ▪ Specialized lab supplies and infrastructure are needed ▪ Blood samples must be processed within 8–30 hours after collection ▪ IGRA cannot replace TST in low-resource areas because it is an expensive test with similar performance when compared with other tests ▪ Limited data on use for children under 5 years, people recently exposed to TB, and immuno-compromised individuals ▪ IGRA and TST cannot predict the risk of infected people in developing active TB disease ▪ IGRA and TST should not be used for the diagnosis of active disease ▪ Adequately trained staff are needed ▪ IGRAs are not reliable when it comes to repeated testing

Sources: World Health Organization. Implementing TB diagnostics. World Health Organization. 2015 Geneva: WHO policy framework. Available from http://apps.who.int/iris/bitstream/10665/162712/1/9789241508612_eng.pdf?ua=1 (Accessed 2016 July 29)

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Activists should:

- advocate against the use of IGRA and TST for the detection of active TB as per WHO recommendations.
  - A particular IGRA (Qiagen's QuantiFERON-TB Gold) was misused by the private sector in India for the detection of active TB after the tests were banned in India for the diagnosis of active TB. Activists responded by writing to the diagnostic company for responsible marketing of the IGRA so that doctors in India only use the IGRA for the diagnosis of latent TB;
- advocate for the availability of preventive therapy per WHO guidance regardless of implementation of IGRA or TST;
- advocate for more research into who is at risk of progressing from latent TB to active disease, and how to identify those people.

NONAPPROVED WHO TESTS FOR THE DETECTION OF ACTIVE TB

Serological tests

All currently available blood-based, or serological (also called serodiagnostic), tests for the detection of pulmonary TB and EPTB are not WHO-recommended for use. In fact, WHO issued a negative recommendation (meaning "do not use this test" for serological TB tests).[48] The reason leading to the lack of endorsement of the blood-based tests for active TB is that such tests have low sensitivity (high false negative results) and low specificity (high false positive results). Activists advised the banning of such tests for use in India, particularly in the private sector, and such efforts successfully led to India banning the tests.

Activists should:

- monitor for use of banned of serological tests for the diagnosis of TB in their settings;
- create accountability for inappropriate use or marketing of these tests by laboratories, companies;
- call for governmental oversight to protect against use or marketing of these tests.

IGRA for active disease

As stated above, WHO does not recommend the use of IGRAs for the diagnosis of active TB.

Activists should:

- urge governments and the private sector to prevent the use of IGRA for the diagnosis of active TB.

Genedrive

A new NAAT has been developed for the detection of TB and rifampin-resistant TB; however, the sensitivity (zero percent in smear negative samples) and specificity (45.6 percent in smear positive samples) is lower than smear microscopy.[49] Therefore, Genedrive yields high false negative results, misses many cases, and offers no advantage over smear microscopy. The test performs poorly in detecting TB in smear-negative and culture-positive samples.[50] Genedrive, manufactured by the company Genedrive (formerly called Epistem), does not meet WHO requirements due to the low accuracy of the test.

Activists should:

- strongly discourage the use of Genedrive in all settings, and encourage the Indian regulatory authority (the Drugs Controller General of India) to reverse marketing approval for Genedrive in India;
- push Genedrive to halt marketing until reputable, peer-reviewed data are published to support its use.

GLOSSARY

algorithm:	a set of rules to be followed; in the case of tuberculosis diagnostics, as many tests can be required to guide appropriate treatment, algorithm refers to a flow for when to introduce certain tests in a given population
antibody:	a protein in the blood that is part of the body's immune response and is made to fight a specific antigen
antigen:	a foreign substance that makes the body have an immune response, which is usually characterized by the production of antibodies by the body
biomarker (biological marker):	any objective indication of medical state that can be measured accurately and reproducibly.[51] Common examples of biomarkers include CD4 cell count for indicating progression of HIV's effect on the immune system and HIV viral load, which is a good biomarker for HIV treatment efficacy. TB lacks good biomarkers for disease, treatment efficacy, and cure.
biosafety:	safety level in the laboratory, usually required to prevent the spread of bacteria and or virus in the laboratories. The more dangerous the virus or bacteria, or the less secure the test to look for them, the higher the safety level required.
false negative:	incorrect diagnosis of not having a disease or infection when the individual does indeed have the disease or infection (low sensitivity leads to false negatives, which can result in not receiving the right and enough treatment or "undertreatment")
false positive:	incorrect diagnosis of having the disease or infection when the individual does not indeed have the disease or infection (low specificity leads to false positives, which can result in receiving treatment when one does not have the disease or "overtreatment")
fluorescence:	the property of absorbing light of shorter wavelengths and of giving off light of longer wavelength; in TB diagnosis, fluorescence can improve the sensitivity of microscopy—using fluorescent molecules to stain a TB sample helps to make the *Mycobacterium tuberculosis* more visible and therefore easier to detect.
gastric lavage:	cleaning out the contents of the stomach; in TB, this is a useful (but an invasive) technique for finding *Mycobacterium tuberculosis* in people who cannot produce sputum, especially children
genotypic testing:	testing that examines the genetic make up (DNA or RNA) of an organism; in TB, genotypic tests such as NAATs can identify both the presence of TB and certain types of drug resistance.
hemoptysis:	coughing up blood
high throughput:	describes a technique or technology that can process a high number of samples from the body
invasive:	describes tests or procedures that introduce instruments or substances into the body, and are usually uncomfortable or carry risk
isolates:	separated samples
LAM:	lipoarabinomannan (an antigen or protein in the cell wall of *Mycobacterium tuberculosis* which is present in people with active tuberculosis disease)
lesion:	an area in the body which has suffered damage through an injury or disease; in TB, for example, when *Mycobacterium tuberculosis* bacteria enter the lungs, the body's immune response tries to wall it off, creating lesions (also sometimes called granulomas) which can kill tissue. This damage can be detected in X-rays.

memory T cell:	a type of antibody which can overcome a virus or bacteria by 'remembering' the strategy used to eliminate previous infections
microbiological confirmation:	a test which determines presence of infection or disease by looking for the presence of the infection-causing organisms (such as bacteria), rather than a reaction to it. In TB, for example, NAAT, culture, and microscopy are all forms of microbiological confirmation, whereas X-ray is not.
NAAT:	nucleic acid amplification test, a test that amplifies and detects genetic material; many tuberculosis tests explained in this guide detect tuberculosis by detecting the genetic material of *Mycobacterium tuberculosis*
phenotypic testing:	testing that determines the presence of a particular trait by looking at the physical expression of the organism, rather than its genetic material. In TB, phenotypic testing refers to using culture to determine drug susceptibility.
rule in:	to determine that someone has a disease; a highly specific test with positive results 'rules in' a disease, but a negative result can still require further testing, as the test is not sensitive enough to (for example, LAM testing)
rule out:	to determine that someone does not have a disease; a highly sensitive test with negative results 'rules out' a disease, but a positive result may require further testing to confirm presence of TB (e.g. symptom screening)
sensitivity:	the proportion of people with a disease who are correctly identified by a diagnostic test as having the condition (disease). Low sensitivity leads to a high number of false negative results. A sensitive test helps to rule out disease (when the result is negative).
specificity:	the proportion of people without a disease who are correctly identified by a test as not having the condition. Low specificity results in a high number of false positive cases. A very specific test (when the result is positive) helps to rule in disease.
sputum:	a mixture of saliva and mucus coughed up from the respiratory tract, typically as a result of infection or other disease and often examined to help diagnose TB
sputum induction:	a procedure for bringing out sputum in people who need coughing it up; the procedure introduces vaporized salt water into the patient's airways to create extra moisture in the lungs, which loosens the sputum so it may be coughed up more easily.
T cell:	a type of white blood cell produced in the body's thymus gland and involved in immune response. CD4 cells, which are attacked by HIV, are a type of T cell. T cells also play a role in the immune response to TB, although it is unclear what an effective immune response to TB involves.
triage test:	a test that includes separating patients according to severity of disease or condition so as to prioritize who to send for further diagnosis or treatment. In TB, chest X-ray is usually used as a triage test when it is available, as it can identify who may need to go on to microbiological confirmation. Also known as a screening test or tool.
yield:	the amount produced. In TB, yield is often used to refer to the proportion of people identified as having TB when screening takes place; high yield refers to a high proportion of people with TB out of the total screened, and can demonstrate efficient use of resources.

Summary of TB diagnostic tests

Test name	Sensitivity	Specificity	DST	Speed	Point-of-care	No onerous lab/ biosafety requirements	No reagents/ supplies	Non-sputum based	Useful in special populations		
									Children	HIV	EPTB*
TESTS for TB DISEASE											
GeneXpert MTB/RIF	✓	✓	✓ RIF	2 hours		✓	Needs cartridges	(can use some other samples)	✓	✓	✓
MGIT liquid culture	✓	✓	✓	7–14 days for smear-positive samples 4 weeks for smear-negative samples			Needs liquid media	(can use some other samples)	✓	✓	✓
Solid culture	✓	✓	✓	2 months			Needs solid media	(can use some other samples)	✓	✓	✓
Line probe assay—first-line	✓	✓	✓	1–2 days (depends on batching)			Needs parts for the instrument	(can use some other samples)	✓	✓	
Line probe assay—second-line (MTBRDsl)	✓	✓	✓ LEV OFX KAN AMK CAP ETH	1–2 days (depends on batching)			Needs parts for the instrument	(can use some other samples)	✓	✓	
Chest X-ray	✓	Low				✓	Needs parts for the instrument		✓	✓	
Digital chest X-ray	✓	Medium			✓	✓	Needs parts for the instrument		✓	✓	
Smear microscopy	Low			1 day		✓	Needs parts for the instrument				
LED- automated microscopy	Medium					✓	Needs parts for the instrument				
LAM	Low	✓		25 min	✓	✓		✓ Urine	✓ (HIV+ and CD4 < 100)	✓ (CD4 < 100)	✓ (HIV+ and CD4 < 100)
TESTS FOR TB INFECTION (LATENT TB)											
TST	Medium	Low		48–72 hours		✓		✓ Skin	✓		
Quantiferon	Medium	✓		24–48 hours		✓		✓ Blood			
Elispot	Medium	✓		1 day		✓		✓ Blood			

*EPTB=extrapulmonary tuberculosis

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