

# Approaches to tuberculosis screening and diagnosis in people with HIV in resource-limited settings

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Tuberculosis is the main cause of morbidity and mortality in people living with HIV/AIDS worldwide. Early diagnosis and treatment is essential to addressing the dual epidemic of tuberculosis and HIV. Increasing recognition of the importance of integrating tuberculosis services—including screening—into HIV care has led to global policies and the beginnings of implementation of joint activities at the national level. However, debate remains about the best methods of screening for pulmonary tuberculosis among people living with HIV/AIDS in resource-limited settings. Mycobacterial culture, the gold standard for tuberculosis diagnosis, is too slow and complex to be a useful screening test in such settings. More widely available methods, such as symptom screening, sputum smear microscopy, chest radiography, and tuberculin skin testing have important shortcomings, especially in people living with HIV/AIDS. However, until simpler, cheaper, and more sensitive diagnostics for tuberculosis are available in peripheral healthcare settings, a strategy must be developed that uses current evidence to combine available screening tools.

## Introduction

The global HIV pandemic has presented an immense challenge to the diagnosis, treatment, and prevention of tuberculosis—the leading cause of morbidity and mortality in people living with HIV/AIDS. Programmes to combat the two diseases are increasingly working together to rapidly implement approaches to tuberculosis screening with the current infrastructure and availability of diagnostic tests. However, recent global data suggest that implementation of screening for tuberculosis in HIV care settings is unacceptably low.<sup>1</sup>

Early and accurate tuberculosis diagnosis is needed to improve treatment outcomes for individual patients and to reduce transmission. The high early mortality rates documented in people with both HIV and tuberculosis<sup>2,3</sup> underscores the need for rapid diagnostic tests. In addition, higher rates of smear-negative and extrapulmonary tuberculosis among people with HIV<sup>4</sup> call for more sensitive, simple assays that can be used in peripheral health centres.

Two important therapeutic interventions are available to curb tuberculosis incidence among people with HIV: isoniazid preventive therapy (IPT) and antiretroviral therapy (ART). IPT reduces the risk of tuberculosis in people living with HIV/AIDS by up to 62% in patients with a positive tuberculin skin test.<sup>5,6</sup> However, concerns about inadequate tuberculosis screening before starting treatment, with the resulting risk of isoniazid monotherapy and resistance, have hampered scale-up of IPT programmes.

ART lowers the incidence of tuberculosis in people living with HIV/AIDS in resource-limited settings.<sup>7</sup> Although some patients with undiagnosed tuberculosis have immune reconstitution inflammatory syndrome when starting ART, it is rarely fatal and can be treated. Nonetheless, the syndrome can cause substantial morbidity, so screening for tuberculosis is an integral component of the assessment of patients starting ART.<sup>8</sup>

Tuberculosis screening therefore serves two main purposes: to identify patients with tuberculosis (case

detection) and to exclude active tuberculosis so patients can safely start other treatments. When evaluating a diagnostic test, one must take into account its sensitivity and negative predictive value (in part a function of disease prevalence; figure). In this paper we review the most widely available methods for tuberculosis screening, recognising that access to even these tests will vary across settings. Although extrapulmonary tuberculosis is a substantial source of morbidity and mortality and poses a serious diagnostic challenge in people living with HIV/AIDS, we focus on the evidence base for methods used primarily in the diagnosis of pulmonary tuberculosis.

## Imperatives for and problems with screening

Tuberculosis is the leading cause of morbidity and mortality in adults infected with HIV worldwide.<sup>9</sup> Indeed the so-called cursed duet<sup>10</sup> of infection with HIV and *Mycobacterium tuberculosis* is generating a threat to human health of unparalleled proportions.<sup>11</sup> Among the 9.2 million patients diagnosed with tuberculosis in 2006, about 700 000 (7.7%) were HIV-positive,<sup>12</sup> with the highest rates of coinfection in sub-Saharan Africa.<sup>1</sup> The interplay of these two diseases means that tuberculosis can spread rapidly<sup>13–15</sup> and recur frequently among people living with HIV/AIDS.<sup>3,16</sup> Tuberculosis progresses from latent to active infection more quickly in people living with

		Presence of disease (confirmed by gold standard)		
		Disease present	Disease absent	
Test outcome	Positive	True positive (A)	False positive (B)	→PPV A/(A+B)
	Negative	False negative (C)	True negative (D)	→NPV D/(C+D)
		↓ Sensitivity A/(A+D)	↓ Specificity D/(B+D)	

Figure: Calculation of sensitivity, specificity, and predictive values  
PPV=positive predictive value. NPV=negative predictive value.

Lancet Infect Dis 2009;  
9: 173–84

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### Panel: Guidelines for tuberculosis screening in people living with HIV/AIDS

#### American Thoracic Society/Centers for Disease Control and Prevention/Infectious Disease Society of America<sup>19</sup>

- Assess for tuberculosis or latent infection in all patients with newly diagnosed HIV; include symptom screening (cough 2–3 weeks, fever, night sweats, weight loss, or haemoptysis; or unexplained cough and fever), history of exposure, physical examination, and chest radiography; tuberculin skin test with 5 mm induration cut-off recorded as positive
- Annual tuberculin skin test after HIV diagnosis
- When CD4 <200 cells/ $\mu$ L and tuberculin skin test negative, patients should have repeat tuberculin skin test after initiation of ART and immune reconstitution

#### British Thoracic Society/British HIV Association<sup>20</sup>

- Do not recommend tuberculin skin test for screening in people living with HIV/AIDS
- Screening should involve case finding by physicians caring for people living with HIV/AIDS

#### WHO<sup>21</sup>

- Screening of people living with HIV/AIDS should involve intensified case finding in all HIV testing and counselling centres with, at least, a simple questionnaire
- A referral system should be established between HIV counselling and testing and tuberculosis diagnostic and treatment centres
- Tuberculosis case-finding should be done on a regular basis

HIV/AIDS than in otherwise healthy people<sup>3</sup> and speeds up the natural course of HIV disease.<sup>3,13–16</sup>

Despite a clear mandate for tuberculosis screening in people living with HIV/AIDS there are many clinical and programmatic challenges. Tuberculosis presents atypically in many cases, with some of the classic signs and symptoms being absent and a high rate of extrapulmonary or smear-negative pulmonary tuberculosis.<sup>4,17</sup> Furthermore, tuberculosis and HIV/AIDS programmes are rarely well-integrated.<sup>18</sup> With rapid scale-up and decentralisation of HIV prevention, care, and treatment services, HIV programmes need to meet the challenge of screening and diagnosing tuberculosis among people with HIV. Therefore, simple tools and standard guidance on approaches to tuberculosis screening and diagnosis in people with HIV are urgently needed, especially in resource-limited countries.

Increased collaboration between disease programmes is necessary for effective screening for tuberculosis in people living with HIV/AIDS. However, current guidelines differ in what that screening involves. The panel outlines guidelines from three international bodies. The algorithm devised by US organisations is applicable

to low-incidence tuberculosis settings, but its usefulness in resource-poor countries and in communities with high HIV seroprevalence is unclear.

WHO guidelines recommend screening all people living with HIV/AIDS with intensified case finding. Intensified case finding extends the conventional DOTS (directly observed therapy short-course) strategy of passive case detection, whereby patients self-present when ill, to active case finding for tuberculosis among people who are in HIV care and treatment settings. However, despite these guidelines, intensified case finding has not been widely implemented, and where it is used, infrastructures do not always enable subsequent definitive diagnosis and treatment.<sup>1</sup> 115 countries have policies recommending intensified case finding, but only 44 reported that it was actually taking place. Less than 1% of people with HIV worldwide were screened for tuberculosis in 2006.<sup>1</sup> The lack of an internationally standardised screening approach is a potential barrier to effective screening.

### Available screening tools

#### Symptom screening

In 2004, WHO recommended that all people living with HIV/AIDS should be regularly screened for tuberculosis.<sup>21</sup> Although there is no clear consensus on what screening involves, most experts agree that it should begin with simple, structured questioning for signs and symptoms of tuberculosis. People with suspected tuberculosis should have more definitive diagnostic testing (eg, sputum smear for acid-fast bacilli, chest radiography, and sputum culture). Cheap and easy to use, symptom screening can be done by all levels of health workers and in the most peripheral health-care settings.

We reviewed several studies related to symptom screening. Their diverse findings show that debate remains on the questions to include in a screening questionnaire (table 1).<sup>22–31</sup> The studies were done in different settings and used different gold standards for assessing sensitivity and specificity. Many national programmes advocate screening for cough alone, since this is the most important and specific symptom of tuberculosis.<sup>25,32</sup> This approach might make public-health sense, because cough suggests patients are infectious (coughing expels respiratory droplet nuclei carrying bacilli) and is effective in screening of a general population.<sup>33,34</sup>

But cough may not be a presenting symptom of tuberculosis in people living with HIV/AIDS. A study in Cambodia at an HIV-testing centre assessed a panel of open-ended questions aimed at eliciting different symptoms.<sup>26</sup> Cough for more than 3 weeks had a sensitivity for tuberculosis of only 55% in people living with HIV/AIDS. A combination of symptoms proved more useful for screening; sensitivity was 100% if patients reported any one of weight loss, fever, or haemoptysis. Unfortunately this same triad had a

specificity of 20%, thus illustrating a fundamental problem of screening with only a symptom-based approach.

In a study in Addis Ababa, Ethiopia, among patients with newly diagnosed HIV, screening for cough alone—of any duration—missed 56% of patients with tuberculosis confirmed by a positive sputum culture. By contrast, screening for any three symptoms—cough, fever, or night sweats—identified 77% of patients with tuberculosis.<sup>23</sup> Another study that screened miners with HIV found the combination of night sweats, cough, or measured weight loss had a sensitivity of 75% and a specificity of 67%.<sup>22</sup> The negative predictive value of this combination of symptoms was 98%, an important consideration when looking to exclude tuberculosis disease in patients starting ART or IPT. However, the negative predictive value is directly related to disease prevalence, so making inferences about the performance of a tool across settings must be done with this in mind. Nevertheless, many studies outlined in table 1 suggest

that a combination of symptoms has far better sensitivity and negative predictive value than does any single symptom.

Sensitivity and specificity are also dependent on other factors, such as age or stage of HIV.<sup>22,26,35</sup> Day and colleagues<sup>22</sup> showed that stratification of screening algorithms by CD4 cell count substantially improved specificity. Certainly HIV is a major modifier of the clinical stage of tuberculosis; when the CD4 count is lower, symptoms may be atypical,<sup>36</sup> minor,<sup>31</sup> or completely absent.<sup>35,37</sup> Where CD4 cell counts are not available, the WHO clinical staging of HIV disease may be a useful proxy in risk stratification of people living with HIV/AIDS for concomitant tuberculous disease.

The addition of more symptoms to the screening criteria increases sensitivity, but at a cost: with a more thorough questionnaire, the specificity declines, because of the higher proportion of false-positive results. The advantage of this approach is that fewer cases of tuberculosis will be missed. However, in resource-limited

	Sample size and setting	Tuberculosis case definition (gold standard)	Number of tuberculosis cases	Sensitivity and specificity
Day (South Africa) <sup>22</sup>	899 gold miners with HIV in a tuberculosis preventive therapy clinic	Clinical features or CXR, plus either SCx(1) or response to tuberculosis treatment	44 (5%)	Cough ≥3 weeks: sensitivity 14%, specificity 96% At least one of night sweats, cough, measured weight loss: sensitivity 75%, specificity 67%
Shah (Ethiopia) <sup>23</sup>	438 people with HIV in an urban VCT centre	SCx(1) or AFB(1)	32 (7%)	Cough of any duration: sensitivity 44%, specificity 76% At least one of cough, fever, or night sweats: sensitivity 77%, specificity 57%
Kimerling (Cambodia) <sup>24</sup>	441 people with HIV in an urban home-care network	SCx(1)	41 (9%)	Cough >3 weeks: sensitivity 65%, specificity 33% At least one symptom of tuberculosis: sensitivity 95%, specificity 10%
Mohammed (South Africa) <sup>25</sup>	129 people with HIV referred for tuberculosis preventive therapy trial	Clinical features or CXR, plus SCx(1); clinical diagnosis with response to treatment	11 (9%)	Cough >2 weeks: sensitivity 82%, specificity 88% At least two of weight loss, cough, night sweats, or fever: sensitivity 100%, specificity 88%
Chheng (Cambodia) <sup>26</sup>	496 people attending a rural VCT center	SCx(1) or AFB(2)	29 (6%), 20 with HIV	Cough >3 weeks: sensitivity 55%, specificity 59% At least one of fever, haemoptysis, or weight loss: sensitivity 100%, specificity 20%
Wood (South Africa) <sup>27</sup>	762 people (174 with HIV) attending an urban primary care clinic	AFB(2); AFB(1) and SCx(1); SCx(2) with matching genotype	12 (2%), nine with HIV	Cough of any duration: sensitivity 17%, specificity 82% At least two of cough, night sweats, anorexia, or weight loss: sensitivity 25%, specificity 78%
Samb (Tanzania, Burundi) <sup>28</sup>	182 hospitalised patients (130 with HIV) investigated for smear-negative tuberculosis	SCx(1), gastric lavage, or bronchoalveolar lavage (Tanzania); Clinical and radiographic response to treatment (Burundi)	41 (23%), 30 with HIV	Cough >3 weeks: sensitivity 80%, specificity 67% At least two of cough >21 days, chest pain >14 days, absence of expectoration, and absence of shortness of breath: sensitivity 85%, specificity 67%
Espinal (Dominican Republic) <sup>29</sup>	200 HIV-positive compared with 200 HIV-negative controls matched for age and sex from an urban VCT centre	At least three of SS, CXR, tuberculosis contact, TST, or response to treatment	39 (10%), 29 with HIV	Overall, chronic cough associated with seven-times greater risk of tuberculosis; 85% of tuberculosis cases had at least one of prolonged fever, weight loss, chronic cough, anorexia, or night sweats
Burgess (Haiti) <sup>30</sup>	1327 people (474 HIV-positive) at an urban VCT centre	At least two of SS, CXR, AFB(1), or SCx(1); SCx-negative must show clinical response to treatment	76 (6%), 50 with HIV	Odds of tuberculosis two-times greater among those with weight loss (p=0.011); sensitivity and specificity of individual symptoms not specified
Corbett (Zimbabwe) <sup>31</sup>	4668 people (874 HIV-positive) from occupational health clinics	AFB(2) or SCx(2); AFB(1) or SCx(1), plus CXR; CXR with response to treatment	27 (0.6%), 13 with HIV	Of people with HIV, 69% reported symptoms at time of screening; sensitivity and specificity of individual symptoms not specified

VCT=voluntary counselling and testing. CXR=chest radiography suggestive of tuberculosis. SCx=positive mycobacterial sputum culture (number positive). AFB=positive sputum smear result for acid-fast bacilli (number positive). SS=symptom screening. TST=positive tuberculin skin test result.

**Table 1: Studies of symptom screening for tuberculosis in people living with HIV/AIDS**

	Sample size and setting	Methods compared	Gold standard	Findings
<b>Fluorescence microscopy</b>				
Kivihya-Ndugga (Kenya) <sup>48</sup>	993 suspected cases (417 HIV-positive) at an urban chest clinic	Fluorescence microscopy vs ZN	Löwenstein-Jensen culture	Fluorescence microscopy sensitivity 73% vs ZN sensitivity 36%; specificity 100% for both methods
Prasanthi (India) <sup>49</sup>	200 suspected cases (31 HIV-positive) at an urban tuberculosis clinic	Fluorescence microscopy vs ZN	Clinical features and positive CXR	Fluorescence microscopy more sensitive than ZN (26% more cases detected)
<b>Sputum processing techniques</b>				
Habeenzu (Zambia) <sup>50</sup>	488 suspected cases at an urban, hospital-based clinic in a setting of high HIV prevalence	Bleach vs ZN	Fluorescence microscopy	Bleach sensitivity 76% vs ZN sensitivity 43%; specificity 100% for both methods
Bruchfield (Ethiopia) <sup>51</sup>	509 suspected cases at an urban, hospital-based outpatient clinic; 96 (75%) of 168 HIV-positive	Bleach vs ZN	Löwenstein-Jensen culture	Bleach sensitivity 50% vs ZN sensitivity 39%
Gebre (Ethiopia) <sup>52</sup>	100 sputum samples collected from suspected cases at an urban tuberculosis centre in a setting of high HIV prevalence	Bleach vs ZN	Löwenstein-Jensen culture	Bleach sensitivity 70% vs ZN sensitivity 31%; specificity 100% for both methods
Wilkinson (South Africa) <sup>53</sup>	166 suspected cases in a rural hospital in a setting of high HIV prevalence	Bleach vs ZN	Löwenstein-Jensen culture	Bleach sensitivity 44% vs ZN sensitivity 43%; specificity >95% for both methods
<b>Reduced number of smears</b>				
Hirao (Nigeria) <sup>54</sup>	224 suspected cases (106 HIV-positive) at four urban hospital clinics	Same day (spot-spot) vs standard (spot-morning-spot) approach	BACTEC 960 MGIT culture	Same-day sensitivity 56% vs standard sensitivity 58%; specificity 99% for both methods
Cambanis (Ethiopia) <sup>55</sup>	243 suspected cases attending outpatient clinic in a setting of high HIV prevalence	Same day (spot-spot) vs standard (spot-morning-spot) approach	ZN	Same-day sensitivity 87% vs standard sensitivity 89%; specificity 100% for both methods
Crampin (Malawi) <sup>56</sup>	1992 suspected cases (of those with confirmed tuberculosis 165 [57%] of 289 tested were HIV-positive)	Same day (spot-spot) vs standard (spot-morning-spot) approach	Löwenstein-Jensen culture	Same-day sensitivity 69% vs standard sensitivity 70%; specificity 98% for both methods
Harvell (USA) <sup>57</sup>	143 cases (23 HIV-positive) at an urban hospital	Serial smear assessment	BACTEC 460 Mycobacterial Radiometric Broth culture	Three sequential smears needed to reach 100% sensitivity; no increase in yield with serial smears among HIV-positive patients

CXR= chest radiograph findings suggestive of tuberculosis. MGIT=mycobacterial growth indicator tube. ZN=Ziehl-Neelsen method.

**Table 2: Studies of sputum-smear assessment for tuberculosis in people living with HIV/AIDS**

settings, where more additional tests may not be available (eg, sputum smear microscopy or chest radiography), higher specificity may be preferable to avoid overtreatment and undue burden on DOTS programmes. Trade-offs between sensitivity and specificity are therefore common.

In summary, although no current consensus exists, cough alone seems a poor screening method in people living with HIV/AIDS. Use of cough as the only screening symptom is also problematic because there are cultural nuances to the term, and pulmonary diseases other than tuberculosis can cause a cough in people living with HIV/AIDS. The recommendation of cough for more than 2 weeks as the entry criteria into the WHO algorithm for the diagnosis of smear-negative tuberculosis in people living with HIV/AIDS,<sup>38</sup> should be reconsidered in view of available data. An ideal approach may involve a sensitive test in concert with a cost-effective diagnostic work-up to exclude false-positives. Further research in settings with high HIV prevalence is needed both to validate questions included in tuberculosis screening and to determine the best frequency of screening among people living with HIV/AIDS.

### Sputum smear microscopy

After symptom questionnaires, sputum-smear microscopy is the simplest available diagnostic method and has been the mainstay of tuberculosis diagnosis for the past 100 years and a key technique in the DOTS strategy. Traditionally using direct Ziehl-Neelsen staining of sputum samples, sputum-smear microscopy is a highly specific, fast, and cheap method of identifying the most infectious patients. Nonetheless, in this era of increasing HIV prevalence, the sensitivity of sputum-smear microscopy is diminished and interest is growing in ways to improve it.

Conventional microscopy with Ziehl-Neelsen staining requires at least 10 000 organisms per mL of sputum and therefore performs poorly in people coinfecting with HIV who are likely to have smear-negative pulmonary or extrapulmonary disease. In one review, the sensitivity of direct smear microscopy was 31–80%<sup>39</sup> and was lowest in areas with high HIV seroprevalence and in routine conditions. Smear-negative pulmonary tuberculosis in people living with HIV/AIDS, particularly in low-income countries, is a substantial epidemiological problem, accounting for 24–61% of tuberculosis in people with

HIV;<sup>4</sup> although the exact size of the problem may be underestimated. In areas with high HIV prevalence, smear-negative tuberculosis also has much worse prognosis,<sup>40–42</sup> in part due to delays in tuberculosis diagnosis and treatment initiation.<sup>43,44</sup>

#### *Fluorescence microscopy*

An alternative to Ziehl-Neelsen staining is examination of acid-fast bacilli with fluorescence microscopy, which has comparable specificity but is about 10% more sensitive.<sup>42,45,46</sup> Fluorescence microscopy also increases the number of sputum samples that can be read in a given time, because fewer fields must be examined under the microscope, and might, therefore, be more cost-effective than Ziehl-Neelsen staining and a good diagnostic method in resource-limited settings.<sup>47</sup>

Studies have compared fluorescence microscopy with Ziehl-Neelsen smear in people infected with HIV (table 2). A study in Kenya challenged conventional wisdom that fluorescence microscopy is too expensive for use in resource-limited countries; the increased sensitivity of the test meant that fewer samples were analysed, reducing costs, and expediting diagnosis.<sup>48</sup> People with HIV with suspected tuberculosis were less likely to be smear-positive than those not infected with HIV, and, therefore had a greater benefit from fluorescence microscopy than from Ziehl-Neelsen staining (absolute difference in sensitivity 37%). A study in India reported an incremental yield of 26% for fluorescence microscopy over Ziehl-Neelsen staining for people also infected with HIV.<sup>49</sup> The need for electricity and concerns about the stability of reagents used in fluorescence microscopy in rural settings have brought the practicality of its use into question;<sup>58</sup> however, the Foundation for Innovative New Diagnostics has been working closely with partners to support low-cost access to light-emitting diode (LED) fluorescence microscopy, which has a reported lifespan of more than 50 000 h.<sup>59</sup>

#### *Bleach and smear method*

Another method of improving sputum microscopy is the bleach and smear method. Sputum is chemically liquefied then concentrated by centrifugation or sedimentation. The best-established method uses sodium hypochlorite (NaOCl)—household bleach—to liquefy the sputum. The reagent is almost universally available, the entire process can be done without expensive equipment, and disinfectant properties of bleach increase biosafety.

Several studies report that bleach processing of sputum increases the sensitivity of direct Ziehl-Neelsen staining as a diagnostic method (table 2). Although much of the research was done in high-income countries with low HIV seroprevalence,<sup>39</sup> studies from low-income countries have also shown greater sensitivity than with Ziehl-Neelsen staining,<sup>50,52,53,60</sup> even

among people with HIV.<sup>51,61</sup> A recent systematic review included two studies specifically in people with HIV, the results of which were equivocal—one study showed an increase in sensitivity of bleach and smear, the other did not.<sup>39</sup> The main disadvantages of the technique are long processing time and lack of a standardised method.<sup>51</sup> Nevertheless, the technique shows some promise, particularly in peripheral health centres in resource-limited countries.

#### *Reduced number of sputum smears*

Whether collection and testing of three consecutive smears (spot-morning-spot) over 2 days is needed for screening is a matter of contention, especially where prevalence of tuberculosis is high. Reducing the number of smears—with patients giving two samples on 1 day rather than three samples over 2 days—could decrease the burden on patients and laboratories, and thus improve the sensitivity and specificity of direct microscopy. In a study in Malawi, 15% of smear-positive cases dropped out of the diagnostic pathway between submitting specimens and being offered treatment.<sup>62</sup> Cost-effectiveness analysis also supports collection of fewer sputum samples.<sup>63</sup> However, the early morning sample is likely to contain the most bacilli, and might be crucial in people infected with HIV who typically have a low number of bacilli in sputum.

Several studies, not specifically among people living with HIV/AIDS, have concluded that the average incremental yield and increased sensitivity gained by examining a third sputum sample is small.<sup>54,55,64,65</sup> one systematic review estimated that the yield of a third sample is only about 2–5%.<sup>66</sup> In theory, collection of fewer samples from people infected with HIV increases the risk of false-negative results. The authors of the systematic review, however, found three studies with results stratified by HIV status, two of which found no difference in sensitivity in people with and those without HIV.<sup>56,57</sup> WHO recently amended its policy for tuberculosis diagnosis in favour of a two-specimen protocol “in places where a well-functioning external quality assurance (EQA) system exists, where the workload is very high and human resources are limited”.<sup>67</sup>

#### **Sputum culture**

Solid-media sputum culture with Löwenstein-Jensen medium has traditionally been the gold standard for tuberculosis diagnosis in resource-limited settings, although liquid culture is the standard of care in industrialised countries. Culture diagnosis is important for four reasons: it is much more sensitive than microscopy (able to detect as few as ten bacteria per mL of sputum), vital in management of smear-negative tuberculosis; growth of the organisms is necessary for species identification; drug-susceptibility testing requires culture to isolate the organism, crucial since the advent

of multidrug-resistant and extensively drug-resistant tuberculosis; and genotyping of cultured organisms might be useful for investigating tuberculosis transmission or laboratory cross-contamination.

In areas of low HIV prevalence, the sensitivity of conventional culture techniques is 80–85% with a specificity of about 98%.<sup>68</sup> In areas of high HIV prevalence, specificity remains high, but sensitivity is slightly reduced.<sup>69</sup> Solid culture techniques are inexpensive, but some laboratory infrastructure and training are needed. An additional drawback with solid culture is that growth of *M tuberculosis* is slow. One systematic review found that, on average, the mean time to detection with Löwenstein-Jensen cultures was 24 days,<sup>70</sup> but could take up to 6 weeks. In HIV-infected patients, this time might be even longer because of the low number of bacilli in sputum samples.

Fully automated systems that rely on non-radiometric detection of growth, such as the MB/BacT (Biomérieux), BACTEC 9000 (Becton Dickinson), the ESPII system (TREK Diagnostic Systems, Inc), and the mycobacterial growth indicator tube (MGIT; Becton Dickinson), are examples of culture techniques that can speed up diagnosis of pulmonary tuberculosis and improve the sensitivity compared with solid culture methods. These systems involve quite technical procedures for sample processing before changes in gas pressure, carbon dioxide, or oxygen production are used to detect the presence of mycobacteria. However, these systems are much faster than solid cultures. The MGIT system, for example, has a time-to-detection of between 11.6 days<sup>70</sup> and 14.4 days.<sup>71</sup> The sensitivity can be up to 10% higher than that of traditional solid cultures.<sup>70–73</sup> The manual MGIT method offers a reliable, cheaper alternative to the automated MGIT systems.<sup>74</sup> Liquid culture systems can also be applied to drug-susceptibility testing, an increasingly important procedure given rising rates of multidrug resistant tuberculosis worldwide.<sup>70,75</sup>

The main challenge with liquid systems is the high rate of contamination with bacteria and fungi, which cause 5–10% of samples to fail in more sensitive growth media. High rates of laboratory cross-contamination, whereby a positive tuberculosis sample contaminates another specimen, lead to false-positive results. Even in experienced laboratories, cross-contamination rates can be 2.5–10.0%.<sup>76</sup> The need for *M tuberculosis* species identification for all positive cultures creates an additional burden because of the high rate of detection of non-tuberculous mycobacteria in liquid media.<sup>77</sup> Automated techniques unfortunately require resources and expertise rarely available in disease-endemic countries.

One culture technique, the microscopic-observation drug susceptibility assay (MODS), has received much attention because it offers a low-cost and simple liquid culture method, with promising results in resource-limited settings.<sup>78–80</sup> MODS uses liquid media and relies

on microscopic detection of so-called cording growth that is typical of *M tuberculosis*. The time to culture-positivity for MODS (median 7 days) is much less than with automated techniques such as the MBBacT system (21 days) or standard Löwenstein-Jensen techniques (up to 68 days).<sup>81</sup> The cost of MODS (US\$2 per sample), which includes testing for multidrug-resistant tuberculosis, compares favourably with Löwenstein-Jensen culture (\$6) and automated mycobacterial culture (\$52), and further supports the need for greater research and field assessment of this assay in low-resource settings with high HIV prevalence. The manual MGIT method is also an affordable option (\$2–\$10), but without the advantage of testing for drug-susceptibility conferred by MODS.

Culture-based diagnosis of tuberculosis is recommended in *International Standards of Tuberculosis Care*,<sup>82</sup> even in disease-endemic countries. Rapid scale-up of laboratories is needed to ensure access to timely and accurate diagnosis with attention to biosafety standards. Automated methods have not been implemented widely in most resource-limited countries. Even solid culture methods need facilities and expertise that are, in many countries, located in one national reference laboratory or major hospital, if present at all. In response to this urgent need, the Global Tuberculosis Laboratory Initiative has developed a comprehensive strategy that integrates laboratory expansion with health-systems strengthening.<sup>83</sup> Much effort is focused on strengthening of laboratory capacity for tuberculosis diagnostics, from microscopy to culture and new diagnostic techniques. Demonstration projects with the MGIT system underway in high-burden, low-income and middle-income settings have had promising results.<sup>84</sup>

### Chest radiography

Chest radiography has been used as an adjunct in tuberculosis diagnosis, especially for supportive evidence in people with suspected smear-negative tuberculosis, but its role in screening is controversial. Chest radiography is problematic in people living with HIV/AIDS because results depend on intensity and presentation of tuberculosis, which is related to the HIV disease stage.<sup>85,86</sup> With well-preserved immune function, patients might have classic findings of cavitation, upper-lobe disease, or pulmonary fibrosis. But with more advanced HIV, chest radiography can have atypical findings, such as lower-lobe infiltrations or hilar or mediastinal adenopathy.<sup>41,87</sup> The interpretation of chest radiography is further complicated by high rates of other HIV-related pulmonary diseases, such as routine bacterial pneumonia. Consequently, in patients with HIV, no specific radiographic pattern is diagnostic of tuberculosis, and findings on chest radiography can be normal in 25–50% of patients with tuberculosis confirmed with bacteriological testing.<sup>88,89</sup> Therefore, the yield of widespread chest radiography for screening or detecting

	Sample size and setting	Gold standard	Findings
van Cleef (Kenya) <sup>91</sup>	998 suspected cases at an urban, hospital-based chest clinic (270 tested for HIV, 129 positive)	Löwenstein-Jensen culture	559 tuberculosis cases diagnosed. Positive CXR sensitivity 91%, specificity 67%
Samb (Tanzania and Burundi) <sup>92</sup>	182 hospitalised patients (130 HIV-positive) investigated for smear-negative tuberculosis	SCx, gastric lavage, or bronchoalveolar lavage (Tanzania); clinical and radiographic response to treatment (Burundi)	41 tuberculosis cases diagnosed; radiographic finding of adenopathy, reticulonodular infiltrate or patchy infiltrate/consolidation associated with tuberculosis ( $p < 0.001$ )
Burgess (Haiti) <sup>90</sup>	241 suspected cases (128 HIV-positive) at an urban VCT centre	At least two of SS, CXR, AFB, or SCx; SCx-negative must have clinical response to treatment	76 tuberculosis cases diagnosed; positive CXR sensitivity 100%, specificity 63%
Mohammed (South Africa) <sup>95</sup>	129 people with HIV referred for preventive therapy trial	Clinical features or CXR, plus SCx; clinical diagnosis with response to treatment	11 tuberculosis cases diagnosed; positive CXR sensitivity 27%, specificity 96%
Mosimaneotsile (Botswana) <sup>97</sup>	686 people with HIV in preventive therapy pilot programme	None (CXR for diagnosis)	560 (82%) completed CXR process, of whom 536 (96%) were interpreted as healthy; only one (0.2%) tuberculosis case diagnosed solely with radiography
Hawken (Kenya) <sup>98</sup>	2962 people in preventive therapy trial (473 with HIV)	None (CXR considered likely for active disease)	Positive CXR might detect an additional 6% of cases who report no cough
Shah (Vietnam) <sup>99</sup>	876 people with HIV in home-care undergoing annual screening	None (CXR used to identify suspected cases)	191 CXRs show suspected tuberculosis; male sex, older age, and history of tuberculosis associated with higher likelihood of positive CXR

VCT=voluntary counseling and testing. CXR=chest radiography suggestive of tuberculosis. SCx=positive mycobacterial sputum culture. AFB= sputum smear positive for acid-fast bacilli. SS=symptom screening. TST=positive tuberculin skin test result.

**Table 3: Studies of chest radiography for tuberculosis detection in people living with HIV/AIDS**

tuberculosis in people living with HIV/AIDS might be too low and insufficiently specific.<sup>90</sup>

Another limitation of chest radiography is interobserver and intraobserver variation in the interpretation. Several studies over the past 50 years have shown that doctors tend to over-read and under-read chest radiographs.<sup>91–94</sup> Current practice is to reduce the effects of observer error by using a second or third reader and seeking consensus, which is both costly and effort-intensive.<sup>95</sup> Nevertheless, there remains the potential for wide variation between readers ( $\kappa$  0.55–0.84) depending on their level of experience.<sup>96</sup> The atypical presentation of tuberculosis disease in people living with HIV/AIDS further worsens interobserver variability. Finally, the reliability of chest radiography in the diagnosis of tuberculosis is dependent on the quality of the film itself and determined largely by the skill of the radiology technician and the quality of the equipment. In resource-limited settings, good quality chest radiographs are therefore often unavailable in peripheral health centres.

Chest radiography has limited usefulness as a stand-alone screening tool (table 3). In one study of people living with HIV/AIDS in Botswana, only 0.2% of 560 asymptomatic people had tuberculosis diagnosed by chest radiography alone,<sup>97</sup> despite a very high prevalence of tuberculosis in people living with HIV/AIDS. Another study in Malawi showed that screening with chest radiography was less effective and more costly than screening with sputum-smear microscopy.<sup>100</sup>

However, chest radiography might have a role in some settings. A study in Vietnam of people living with HIV/AIDS showed that chest radiography could identify people with suspected tuberculosis for further diagnostic testing.<sup>99</sup> In a study from Kenya, chest radiography was

cost-effective in settings of low tuberculosis prevalence; in settings with greater than 40% prevalence it was more cost-effective to use radiography only in patients with smear-negative sputum results.<sup>91</sup> Both studies show that radiography alone is not very sensitive or specific, and can lead to underdiagnosis and overdiagnosis.

Several studies have shown that, when used with other screening methods, chest radiography can increase specificity of other methods. In a South African study, the negative predictive value of night sweats, new or worsening cough, measured weight loss, or an abnormal chest radiography was 99.2%.<sup>22</sup> High levels of silicosis in the study population might limit the generalisability of these results. Other studies have similarly found that radiography might be useful in the screening of people living with HIV/AIDS when used with symptom screening<sup>98</sup> or smear microscopy.<sup>91</sup> Furthermore, several studies suggest that chest radiography is useful in patients who have negative smear results.<sup>32,101,102</sup>

Several radiological scoring systems, such as the Chest Radiograph Reading System or the four-point scoring system used in van Cleeff and co-worker's study in Kenya, address the problem of interobserver variability. Both scoring systems resulted in better  $\kappa$  scores than expected.<sup>95,103</sup> More work is needed to prove the validity of these scoring systems in settings of high HIV prevalence. However, standardised methods could improve accuracy and consistency of interpretation of chest radiography and might increase its usefulness in screening.

### Tuberculin skin testing

First introduced in 1890, the tuberculin skin test is the oldest diagnostic test for tuberculosis, and is especially useful in children. However, the test is not perfect, and it

	Sample size and setting	PPD cut-off points	Findings
Graham NM (USA) <sup>109</sup>	109 HIV-positive and 151 HIV-negative IDUs in an urban, community-based clinic	≥10 mm, ≥5 mm, and ≥2 mm	HIV-positive less likely to have positive PPD at ≥5 mm cutoff (13.8% vs 25.2%, p=0.02); at ≥2 mm cutoff, no significant difference between groups; significant inverse linear trend with positive PPD rate and CD4 cell count
Mohammed A (South Africa) <sup>25</sup>	129 HIV-positive referred for preventive therapy trial	≥5 mm	PPD ≥5 mm sensitivity 55%, specificity 83% for detecting active tuberculosis; all patients screened were WHO clinical stage 3 or 4
Nachega J (South Africa) <sup>110</sup>	438 HIV-positive in an urban post-natal clinic	≥5 mm	In people with positive PPD ≥5 mm, 11% confirmed with active tuberculosis; median CD4-cell count in patients with tuberculosis was 230 cells/μL; TST-negative people not assessed for tuberculosis, so sensitivity and specificity not calculable
Garcia-Garcia M (Mexico) <sup>111</sup>	1168 subjects (801 HIV-positive) at urban HIV testing centers	≥10 mm, ≥5 mm, ≥2 mm	HIV-positive less likely to have positive PPD at 10 mm, 5 mm, and 2 mm cut-offs (p<0.01 for all cut-offs); positive PPD more likely in BCG vaccinated and CD4 cell count ≥500 cells/μL
Okwera A (Uganda) <sup>112</sup>	94 post-partum women (61 HIV-positive) in an urban hospital	≥3 mm, regardless of HIV status	HIV-negative more likely to have positive TST than were HIV-infected (82% vs 48%, p<0.05); BCG vaccinated, HIV-positive women more likely to have positive TST (75% vs 24%, p=0.05)
Ferreira MM (Brazil) <sup>113</sup>	350 women (87 HIV-positive) in an urban prison	≥5 mm for HIV-positive, ≥10 mm for HIV-negative	No difference in positive PPD by HIV status or BCG vaccination history, or both
Espinal M (Dominican Republic) <sup>29</sup>	200 HIV-positive compared with 200 HIV-negative persons at an urban VCT centre	≥5 mm and ≥10 mm	HIV-positive less likely to have positive PPD at ≥10 mm or ≥5 mm cut-off (p<0.001); BCG vaccination did not affect positive PPD rate in HIV-positive people

VCT=voluntary counselling and testing. PPD=positive purified protein derivative skin-test result. TST=positive tuberculin skin test result. IDU=injecting drug user.

**Table 4: Studies of tuberculin skin testing for tuberculosis in people living with HIV/AIDS**

cannot distinguish latent tuberculosis infection from active disease.

The purified protein derivative used for the tuberculin skin test is composed of antigens found not only in *M tuberculosis*, but also in *Mycobacterium bovis* and other mycobacteria.<sup>104</sup> Hence, in areas where children receive BCG vaccination with a live attenuated strain derived from *M bovis* and where exposure to non-tuberculous mycobacteria is high, the tuberculin skin test might give false-positive results.<sup>105,106</sup> The tuberculin skin test is also compromised by HIV infection, in which immunosuppression can lead to anergy and false-negative test results. HIV infection causes reduced cell-mediated immunity, resulting in more false-negative skin tests and reduced sensitivity of this test. The dose of purified protein derivative, how that is applied to the forearm, and the criteria for interpretation can also affect the sensitivity. For example, weak doses increase the likelihood of false-negative results, whereas strong doses increase the chance of false-positive results.<sup>105</sup> Other problems with the tuberculin skin test as a screening tool include the technical and logistical problems involved. Although inexpensive, the test is not easy to use and variability within and between readers is common. Follow-up is poor in many cases because of the need for patients to return within 48–72 h for test reading.

Despite these limitations, the tuberculin skin test is widely used in initial screening for active tuberculosis and latent infection in developed countries. A positive result identifies which patients should receive isoniazid prophylaxis to reduce progression of latent infection to active disease. Treatment of latent tuberculosis infection in people with HIV is particularly important, because

HIV infection increases the risk of progression to active disease.<sup>107</sup> Treatment of latent tuberculosis infection lowers the risk of active disease in people with HIV by 35–76%.<sup>5,6</sup> This evidence has resulted in targeted use of tuberculin skin tests and treatment programmes for latent tuberculosis infection in people with HIV in developed countries.<sup>106,108</sup>

The role of tuberculin skin tests in settings of high HIV prevalence is less clear (table 4).<sup>109–113</sup> In a study in Mexico, reactivity to purified protein derivative was useful in the diagnosis of tuberculosis only in people with HIV with CD4 counts of 500 cells per μL or higher.<sup>111</sup> At lower counts, reducing the induration cutoff from 5 mm to 2 mm did not produce reactivity comparable to that in people without HIV. These results, which are comparable to those of studies in Uganda,<sup>112</sup> Brazil,<sup>113</sup> and Haiti<sup>114</sup> might reflect high rates of anergy in people with HIV, especially those with CD4 counts of 500 cells per μL or less. As a consequence, a negative tuberculin skin test in a person with HIV should be interpreted with caution. A positive test, however, is evidence for tuberculosis infection, and preventive therapy should be considered for patients without evidence of active disease.

As a stand-alone screening tool in areas of high HIV seroprevalence, tuberculin skin testing has insufficient sensitivity or specificity to be clinically useful. However, combined with other tests, the tuberculin skin test can guide decisions about treatment of disease versus latent infection. Interferon γ release assays, a form of in-vitro, T-cell based assay, are an alternative that can be more specific than tuberculin skin tests for diagnosing latent tuberculosis infection and are being investigated. The expense and expertise needed and lack of evidence to

### Search strategy and selection criteria

Publications on tuberculosis and HIV were identified by searches of Medline and PubMed with terms including, but not restricted to, the following combinations: "tuberculosis", "tuberculosis screening", "human immunodeficiency virus infection", "diagnosis", "purified protein derivative", "tuberculin skin test", and "chest radiography". We focused on articles published between 1966 and 2008. The search was limited to publications in English, but was not restricted by date. Reference lists of these articles were then searched to identify further relevant articles. No language or date restrictions were used. Publications from organisations such as WHO were also included.

support their use in immunocompromised patients or disease-endemic countries mean that, at this stage, their role is unclear.

### Conclusions and future directions

Increasing rates of tuberculosis in people with HIV emphasise the importance and inadequacies of available diagnostic tools. Symptom-screening questionnaires are the mainstay for identification of people who might have tuberculosis in resource-limited settings. This approach is neither standardised nor comprehensive, and more resources are needed to ensure this first step in screening is effective and evidence based. Screening for cough alone is not sensitive enough, so including more symptoms is a practical and simple improvement. Methods to improve sputum microscopy seem promising and possible in many resource-limited settings. However, many people with HIV still might be missed by smear microscopy.

Low specificity, high expense, and interobserver variability preclude widespread use of chest radiography as a stand-alone screening method in resource-limited settings. However, combined with other diagnostic measures, chest radiography can be used effectively in the screening of people living with HIV/AIDS for tuberculosis. The tuberculin skin test lacks sensitivity and specificity for latent tuberculosis infection in people living with HIV/AIDS, and cannot distinguish latent infection from active disease. Sensitive and rapid culture techniques are needed not only to increase case detection and to reduce delays in diagnosis, but also to determine drug resistance. Cost and complexity are real challenges to widespread scale-up of the use of cultures for screening purposes, but this technology certainly warrants greater political commitment and resources for its implementation.

Improving the diagnosis of tuberculosis has been a neglected area of research, in part due to the complex characteristics of *M tuberculosis* itself, the varied host response to infection, and, perhaps, complacency as this disease was nearly eradicated in high-income settings.

However, global resurgence has focused attention on development of better diagnostic tools, especially for use in settings with high-burdens of both tuberculosis and HIV. A point-of-care test that is low-cost, rapid, and sensitive in people with HIV and can be used at peripheral health centres is urgently needed. Regional referral laboratories and networks must be strengthened to provide not only quality assurance for testing methods, but also access to more complex diagnostics as they become available. Any expansion of laboratory capacity in disease-endemic countries must be in concert with attentiveness to biosafety and infection control to protect personnel. Already existing tests can significantly improve detection of tuberculosis in resource-limited settings. The challenge remains to make these available in those places where the co-epidemic is most prevalent and the need for tuberculosis screening in people living with HIV/AIDS most pressing.

#### Conflicts of interest

We declare that we have no conflicts of interest.

#### Acknowledgments

NSS is funded by the Doris Duke Charitable Foundation.

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