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# Strengthening labs and introducing new tools for increased case detection and earlier diagnosis of TB

By Theo Smart

## Key points

- Intensified case finding is one form of active case finding which must be performed routinely in people living with HIV because of their high burden of TB, and the greater risk that they could die of undiagnosed TB. It is also important to conduct active case finding in other communities or households where there is a high risk of TB. By identifying, finding, treating and curing TB in the community, it may also be possible to reduce the risk of continued exposure to TB for people living with HIV.
- Scaling up active case findings efforts will depend on scaling up laboratory infrastructure, either as part of the screening programme itself, or to confirm diagnoses.
- However, old lab tests for TB, such as smear microscopy have a low sensitivity for TB, and would miss many cases particularly among people living with HIV.
- New tests are more sensitive, but there is a controversy about whether it is appropriate to use them for active case finding.
- In HIV-positive people, WHO guidelines recommend the use of the WHO 4 TB symptom screen for active case finding (referred to as intensified case finding (ICF) in people living with HIV).
- This symptom screen asks: "Have you had a cough in the past 24 hours?" "Have you had any fever, night sweats or weight loss?" If the answer for any symptom is "yes" further investigations for TB starting with sputum smear should follow.
- If the answer for all symptoms is "no", TB can be ruled out, but the symptom screen should be repeated monthly if the patient is receiving isoniazid preventive therapy (IPT).
- Some researchers have expressed concern that using the symptom screen may miss some TB cases.
- For instance, it can be very difficult to screen for TB among pregnant women living with HIV, and the symptom screen may need to be adjusted or augmented with laboratory tests to detect some of these cases.

- Preliminary results from a study in Kenya suggest that symptom screens, including the 4 TB symptom screen may miss up to a third of patients living with HIV with signs of TB that can be picked up by state of the art lab tests (liquid culture and GeneXpert).
- TB symptoms screens may miss people with TB that have yet to develop or report their symptoms, so some researchers have suggested that active case finding should rely upon these state of the art lab tests
- One study found liquid culture (which is more sensitive than solid culture) was particularly fruitful in identifying TB in the household contacts of people with active TB. The GeneXpert test is also a highly sensitive test for TB that is being introduced into a number of resource-limited countries. But it should be noted that at present, access to both liquid culture and GeneXpert are rather limited in most of these countries.
- However, there is a controversy over how and whether these tests should be used for TB screening
- Some researchers believe these tests may also be picking up cases where people are transiently infected with TB, or containing an infection that is no longer latent, or recently exposed to TB.
- While a positive culture result in an HIV-positive person with a low CD4 count would justify TB treatment even in the absence of symptoms, the experts we spoke to had differing views on whether it is always appropriate to treat for TB in culture-positive asymptomatic patients.
- It is important for researchers to learn more about what exactly is being detected when patients without TB symptoms test positive by TB culture or by GeneXpert. This could influence the way we think about the best method of screening for TB – and the degree to which expensive new diagnostic technologies are essential for effective TB case finding.
- For the time being the 4 TB symptom screen should be considered the most important element in active case finding. It is essential that TB programmes and facilities focus first on how to do symptom-based screening well.

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## Active case finding and laboratory tests for TB

Large numbers of people suspected of having tuberculosis can be quickly identified through interventions for active case detection, such as household contact tracing, case finding in groups at high risk of TB (such as miners), screening campaigns in communities with a high burden of TB, or intensified case finding in people living with HIV, as described in [the last issue of HATIP](#) – but even the most innovative strategies yielding the highest number of cases will amount to little unless supported by accessible TB diagnostic facilities.

Case finding cannot be divorced from diagnostic capacity. Wherever there are TB screening campaigns, or a shift to more active case finding, laboratory capacity will need to be strengthened with investments in equipment, supply chain management, quality assurance and staff. Good quality assurance must also be emphasised, in order to provide timely and reliable diagnostic services for the increased numbers of TB suspects that will be identified by case finding.

“Overall laboratory capacity, infrastructure and quality need to be greatly improved to assist in the diagnosis and management of HIV-related TB, especially smear-negative, extrapulmonary TB,” according to Global Plan to Stop TB 2006-15, “and to respond to the growing epidemics of drug resistant TB, and the challenge of diagnosing TB in pregnant women and in children. The speed and reliability of TB diagnosis must be improved.”

But the demand on laboratory infrastructure, and lack of clarity about how best to meet it, including how and when to use new diagnostic tools such as GeneXpert, liquid culture, radiography, the urinary LAM antigen test and line probe assays for drug resistance have been topics of discussion at a number of the major scientific meetings during 2011.

One of the controversies regarding laboratory tests is the question of which existing laboratory tests should be used to confirm symptom screening, and whether these tests should be used immediately, instead of symptom screening.

In particular the debate is being stimulated by the availability of GeneXpert MTB/RIF.

GeneXpert is a new diagnostic platform that can identify nucleic acid sequences in the TB genome. It makes PCR-based molecular diagnostic technology available in settings where previously its use would have been impossible, by automating the processes of sample preparation, amplification and detection. A test result can be delivered within two hours that will also show whether the patient has resistance to rifampicin. GeneXpert is [being rolled out in South Africa](#) as the first-line diagnostic test for TB, and several other countries are already beginning to integrate its use into national TB programmes. Nevertheless GeneXpert remains relatively expensive, and its optimal use in the field is still being determined.

## Performance of the WHO 4 TB symptom screen and the lab capacity needed to support TB ICF in people living with HIV

Presentations at the 2011 World Lung Health conference in Lille on intensified case finding on people living with HIV provided particularly profound illustrations of some of the unanswered questions surrounding TB laboratory scale-up. Some of these relate to the implementation and upon the effectiveness of the WHO 4 TB

symptom screen, which asks whether any of the following symptoms are present:

- Current cough (in the past 24 hours);
- Any fever;
- Any night sweats; or
- Any weight loss

Symptom screens are, of course, not specific enough to make a TB diagnosis, but should be sensitive enough to rule one out. A ‘no’ answer to all the symptoms on the WHO 4 TB symptom screen should rule out most cases of active TB, suggesting that it should be safe to provide isoniazid preventive therapy (IPT) to prevent TB.

“For a long time, the first hurdle in considering isoniazid preventive therapy (IPT) was excluding TB disease accurately and efficiently. In this regard, the WHO 4 symptom screening tool is simple and has a high negative predictive value exceeding 95% in high TB prevalence settings,” said Dr Mitesh Desai of the US Centers for Disease Control during one session. “But it needs to be implemented widely and HIV care programmes should be able to document that TB screening is indeed happening at designated times such as the first six months of care [*note – while someone who has none of the 4 TB symptoms receives IPT, to be certain that an active TB case was not missed or has since broken through*] and at least annually depending upon how the country adopts WHO’s guidance. We also need to document that the WHO 4 TB symptoms screening tool is working as well as we think it should, and that if TB does occur while someone is taking IPT, that [the clinician] carefully assess drug susceptibility and response.”

Of note, some of the presentations at Lille suggested that the WHO 4 TB symptom screen by itself misses too many cases and should be replaced or augmented by laboratory diagnostics. These studies are worth reviewing, but it should be noted that these are not performing the 4 TB symptom screen exactly as WHO has recommended (in one case not including ‘any cough’ or repeating it for the first six months in every person who is taking IPT).

For example TB in HIV-positive pregnant women may be unusually difficult to pick up using symptom screening tools, for example, according to a study presented by Dr Rose Kosgei of USAID-AMPATH in Kenya.

Moreover, screening for TB could help reduce the risk of TB immune reconstitution inflammatory syndrome in women who could be put on antiretroviral therapy (ART) and could rule out active TB, allowing TB preventive therapy (IPT) to be started.

But HIV-infected pregnant women have many non-specific complaints such as malaise that are common in early TB, and when they have TB, it is usually smear-negative. Meanwhile, there has been some reluctance to use chest x-rays, for fear this would harm the foetus.

So, the research team at AMPATH decided to conduct a cross sectional study at their clinics in Eldoret and Busia, screening pregnant women living with HIV for TB by using a symptom questionnaire that was somewhat different from WHO’s ICF screen: 1) cough for 2 weeks or more, 2) fever, 3) night sweats, 4) weight loss or failure to gain weight. In addition, those with symptoms including a productive cough provided sputum for smear microscopy and culture, while all consenting participants had a shielded chest x-ray at 14 weeks.<sup>1</sup>

After four months, 187 women met the study criteria. They presented at 27 weeks of gestation and were on average 27 years,

old, with a mean CD4 cell count of 450. Of these, 38 (20%) screened positive for symptoms, while 149 did not.

However, laboratory and radiology found very little evidence of TB disease among those who were symptomatic. Twenty-one of those with symptoms, were smear- and culture-negative. Seventeen others had chest x-ray, only three of which were suggestive of TB (cavitary, milliary, infiltrates).

Oddly, the chest x-ray was suggestive of TB in seven out of the 100 without symptoms (on this symptom screen). Dr Kosgei placed great emphasis on the fact that, had symptom screening been used alone, these cases would have been mistakenly put on IPT. On the basis of chest x-ray alone, only 40% of the entire cohort were considered appropriate candidates for IPT (a third didn't consent to chest x-ray).

"The study did not support the utility of isolated symptom screening in our PMTCT setting. Chest radiography was useful in identification of TB suspects in both symptomatic and asymptomatic women," said Dr Kosgei. "Given the profound risks of TB to the HIV-infected pregnant woman and her subsequent infant, further well designed studies targeted at best screening practices in this specific population are needed."

However, the symptom screen was not the same screen as recommended by WHO (which looks for 'any' cough, and repeats screening to watch for breakthrough cases), and it is possible that the AMPATH team may be placing too much weight upon the reliability of chest-x-ray, particularly in the absence of culture confirmation for any of these cases. Nevertheless, the point that symptom screening needs tailoring or that diagnostic rigour needs to be heightened in pregnant women living with HIV is well worth taking to heart.

## TB symptoms screening for TB ICF versus laboratory based screening and diagnosis

Meanwhile another study in Kenya is seeking to validate WHO's ICF guidelines (and screening tool) regionally.

"There is some language in the WHO Guidelines, some caveats that bear mentioning," said Dr Sean Cavanaugh of the CDC. "First, performance of screening is contingent upon prevalence of disease and other diseases that might present with similar symptoms. The symptoms and reporting can be influenced by regional and cultural factors such as smoking prevalence, which could certainly affect coughing, in the way coughing is reported to clinicians. Regional temperatures might influence the experience of fevers and night sweats, [as could] when patients in the course of their disease register for HIV care and how well they communicate the symptoms with the clinician, all of which could be culturally bound."

"Screening algorithms therefore should be validated in the region before they are to be rolled out, ideally," he said.

Dr Cavanaugh presented preliminary findings of a study that KEMRI and CDC performed attempting to validate and compare three clinical screening methods ruling out TB in HIV-infected persons while evaluating the performance of various diagnostic tests, including new tools such as GeneXpert, to determine the prevalence and incidence of TB among HIV-infected persons in Kenya.<sup>2</sup>

The symptoms screens included:

### The ID-TB/HIV symptom screen

This was based on the ID-TB/HIV study that enrolled approximately 2000 patients from 2004 to 2006 in South-East Asia.

Dr Cavanaugh said this screen is considered to be very robust, as it was based upon the "gold standard" for TB diagnosis (including three cultures of sputum; one of urine, one of blood and one of stool from every patient). Yes to any of the following questions should identify the patient as a TB suspect who should receive a full diagnostic work-up for TB, that would include X-rays and smears):

- Any cough in the past four weeks
- Any fever in the past four weeks
- Drenching night sweats lasting for more than three weeks

### The WHO 4 Symptom TB screen

This is based upon a broad review of ICF studies (as described above).

- Current cough (in the past 24 hours);
- Any fever;
- Any night sweats; or
- Any weight loss

### The Kenya Ministry of Health (MoH) symptom screen

Like many other ministries of health, Kenya's MOH have rolled out their own ICF case-finding algorithm that's based mostly on expert opinion of the disease epidemiology in their country, which was intended to be very sensitive. It used seven elements:

- Cough for 2 or more weeks: This symptom alone prompts an evaluation for TB, with microscopy and X-ray if available
- Fevers for 2 or more weeks
- Night sweats for 2 or more weeks
- Noticeable weight loss
- Contact with known case
- Chest pain or breathlessness
- Swelling in neck, armpit, ABD, joints or groin
- Any of these above symptoms would prompt the clinician to engage in a more detailed interview, and physical exam.

The study enrolled newly registered patients at 15 randomly selected HIV clinics in three northern districts in Nyanza Province, who were seven years old or older and had not received TB therapy in the past year, and who consented to participate. All patients were screened using all three recommended ICF tools. Participants are due to be followed for a full year to assess incidence of clinical disease.

At enrolment, the participants provide three sputum specimens, regardless of symptoms. The first specimen is sent for fluorescent microscopy and culture; the second, a morning specimen, is sent for standard (ZN staining) microscopy, fluorescent microscopy, GeneXpert and culture. Specimen 3, is the second spot specimen – which was either collected on that first day or on the second day when the morning specimen was brought in. All culture results are confirmed by a Capilia TB test and line-probe assay. In addition, as a sub-study, stool and lymph node aspirates are being collected from the subset of patients at the larger hospitals.

A TB case was defined as a patient with M. tuberculosis identified on any culture or nucleic acid amplification test. A patient was defined as being negative for TB if he or she had one sputum culture with no growth, plus another sputum specimen and that was negative by culture or nucleic acid amplification. Participants who



didn't meet either of these definitions were considered not evaluable (these had missing samples or contaminated cultures).

### Lab diagnosis results

Dr Cavanaugh presented data from three of the study's 15 sites (the first phase of the study). 245 patients enrolled into the official study: 224 with known TB status (positive or negative), 21 were not evaluable. At Kisumu District Hospital, the largest site (located in Kisumu city), 22 of the 139 enrolled patients had TB disease with a prevalence of 16%; in Nyahera Health Centre, five out of the 40 enrolled patients, (13%) and at Yala sub-district hospital, six out of 45 patients (13%) were diagnosed with TB. Overall there were 33 cases out of 224 enrolled, for a total prevalence of 15%, in this population.

The diagnoses were made on the following basis:

- GeneXpert, alone: 4 cases (12%)
- Sputum Culture and GeneXpert: 22 cases (66%)
- Sputum Culture alone: 7 cases (21%)

Dr Cavanaugh then presented calculations to determine the incremental yield (IY) from adding culture or GeneXpert to the diagnostic work-up — in other words, how many more cases would be diagnosed if these lab tests were routinely available for diagnosis. For culture, the sample size was 30, as there were only 30 patients who had both two sputum smears examined by fluorescent microscopy, and two evaluable (non-contaminated) cultures. Out of those 30:

- Two fluorescent microscopy smears alone would diagnose only twelve cases (40% sensitivity for fluorescent microscopy);
- If one culture is performed in addition to those two fluorescent microscopy tests, twelve more cases can be diagnosed for a total of 80% of the 30 cases and an IY of 12 or 40% (increased sensitivity for adding one culture).
- Adding one more culture (two cultures total), allowed for four more cases to be identified (for an incremental yield of 13% - four out of 30).
- Together, a laboratory diagnostic work-up of two fluorescent microscopy tests, plus two cultures would diagnose 93% of the evaluable patients with TB; two cases would remain undiagnosed.

Doing the same analysis for GeneXpert yielded the following: again, the two fluorescent microscopy alone diagnosed twelve cases, adding one GeneXpert test which found 10 more cases, adding 33% incremental yield — ten out of 30; running a second GeneXpert added four more cases ( $IY = 4/30 = 13\%$ ). Four cases would be missed using just microscopy and the GeneXpert.

Since the mid-morning specimen was sent for all four tests, it allowed a direct comparison, an 'intra-specimen comparison' of test performance. There were 197 morning specimens with both one culture and one GeneXpert. A number of these were either contaminated cultures (34) or non-tuberculosis mycobacteria (12) on culture, leaving 151 comparable specimens — 16 of which were diagnosed with TB. Of those, 13 cases were identified by both tests. There were two patients that were positive on culture but negative on GeneXpert, and one patient that was positive on GeneXpert but negative on culture.

"There's been some recent discussion about the utility of doing a one-off test. It would certainly make things cheaper for programmes, so we're looking at the utility of a one-off test for TB, using the more expensive technology of culture and nucleic acid

amplification," said Dr Cavanaugh. The study's early results are in the table below, however, it should be noted that the analysis was restricted to evaluable patients who had non-contaminated results — which would clearly affect the utility of a one-off test in practice. Dr Cavanaugh noted that the contamination rate was especially elevated at the beginning of this study but has since started coming down.

### Performance of a single test

Test	Evaluable participants	Sensitivity (%)	Negative predictive Value (NPV) (%)
Single spot culture	206	25/29 (86%)	177/181 (98%)
Single spot GeneXpert	220	22/32 (69%)	188/198 (95%)
Morning culture	168	19/24 (79%)	144/149 (97%)
Morning GeneXpert	185	17/25 (68%)	160/168 (95%)

As for the performance of the screening tools? They all appeared to come up rather short (see below), and would miss *many* cases that would be picked up if all patients were simply providing specimens for culture and GeneXpert.

### Performance characteristics: recommended ICF screenings

Screening Tool	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
WHO (n = 209)	21/31(68%)	100/178 (56%)	100/110 (91%)	21/99 (21%)
ID-TB/HIV (n = 211)	18/29 (62%)	111/182(61%)	111/122(91%)	18/89(20%)
Kenya MoH N = 200	17/25 (68%)	102/175(58%)	102/110(93%)	17/90 (19%)

Dr Cavanaugh noted that the analysis had yet to be merged with data on important clinical variables like chest radiographs, smear grade and CD4 counts.

It is worth mentioning, however, that Kenya's more complicated screen did perform slightly better, suggesting that it may indeed be in the patients and the programme's best interests to adapt the symptom screen to regional characteristics —so that the symptom screen reflects how local people living with HIV describe or report their symptoms.

It should be stressed that these are preliminary interim data with a small sample size, so no firm conclusions should be drawn yet.

"But this analysis suggests that the prevalence of TB in this population is quite high — at least around 16%" said Dr Cavanaugh. "The proportion of patients without symptoms is quite high — about 68% of the TB cases screened positive by four-question screening questionnaire, so about 30% of the TB cases did not report any of the four symptoms that we were investigating, and the negative predictive value of all three guidelines is moderate, at best — though we need to evaluate how chest radiography might influence results."

### Should we move to laboratory screening of all people living with HIV or household contacts?

"Although it is too early to tell, a *single sputum specimen tested by culture, and then possibly by the GeneXpert MTB/RIF assay, may*

have reasonable negative predictive value – and that will be something that we'll be looking at, as our data comes in," Dr Cavanaugh concluded.

He was not the only researcher at the conference concerned about the lack of agreement of the symptom screens with sensitive diagnostic tools such as GeneXpert and liquid culture diagnosed as TB. As noted in the previous HATIP Dr Adrienne Shapiro of Johns Hopkin's University found that the success of an active case-finding strategy was dependent upon the use of TB culture.

"93% of the contacts were diagnosed solely on the basis of TB culture, most were smear-negative. The contacts were largely completely asymptomatic – only 11% had any symptoms. If we relied so much on sputum smear, we would have found very little TB. So this also has consequences for cost and feasibility, of future applications of active case finding or household contact tracing," she said.

One potential implication regards the use of IPT, according to Dr Shapiro.

"The results of this study suggest that there may be a fair amount of asymptomatic culture-positive TB that would be inaccurately categorised as no TB. And these patients are given IPT, which would not be appropriate since they would require four-drug TB treatment. So, the screening policy may need to be adjusted for extremely high incidence settings, or these results taken into account when thinking about how to screen for IPT," she said.

However, this writer has noted that researchers consistently omit to mention one critical aspect of the implementation of the WHO screening tool, which is that screening is meant to be repeated on a monthly basis in anyone who is put on IPT, so that any cases that escape detection before IPT is started should be caught in one of the succeeding months if they are already in routine care. The available clinical evidence suggests these cases respond to standard TB treatment.

It should be remembered that before the implementation of the ICF/IPT policy, clinic staff rarely performed routine screening for HIV in PLHIV. Consequently, there were many opportunities missed: many cases remained un-prevented, undetected, undiagnosed, untreated – leading to increased transmission and mortality.

## Is GeneXpert only capturing cases of reactivation TB?

These data and the discussion at the World Lung Health conference in Lille are part of an ongoing debate that HATIP has made reference to in the past about TB that shows up on sensitive lab tests, even though there are no symptoms. In particular, programmes have cited such data as an excuse not to scale up intensified case finding, or IPT. But there is a controversy about the extent to which the infection picked up by these tests is really asymptomatic active TB.

It should of course be expected that as lab tests become more sensitive, they may detect incipient TB cases that may not be causing the patient significant symptoms.

"Culture-positive asymptomatic TB exists. This has been repeatedly demonstrated in high-quality studies," Professor Annelies Van Rie of the University of North Carolina told HATIP.

As Dr Steve Lawn said at the Union World Lung Conference in Cancun a couple of years back: "Our ideas of latent and active TB are very dated – latent TB infection and TB disease probably do not exist in a binary state. HIV may disrupt granulomas containing latent MTB, then mycobacterial load probably increases before TB

becomes symptomatic," he said, "so we need to think outside that box."<sup>3</sup>

But if these cases are early reactivation disease, it is not clear at what point a growing mycobacterial load begins to cause symptoms, or when people become infectious or whether full TB treatment is truly necessary in all cases.

Even less is known about the clinical course of HIV-negative individuals without symptoms who are smear-negative, but who show evidence of MTB on culture or GeneXpert. Are they infectious and when might they become so?

And another possibility also exists: that this is not reactivation TB, but is rather primary TB (an initial infection) or reinfection (which can happen to people with a latent infection, potentially multiple times).<sup>4</sup> In immune competent individuals, the immune system eventually controls and contains both primary infection and reinfection, but for a while these infections will produce evidence of infection that can be picked up by both liquid culture and GeneXpert (in the case of the latter potentially for quite some time after the infection).

"The GeneXpert detects both live as well as dead organisms," said Dr Kogi Naidoo from CAPRISA, in a question to panellists in a symposia on GeneXpert at the South African AIDS Conference. "What happens if you've got a positive TB diagnosis, by GeneXpert, but your culture comes out negative? What's your advice to people on the ground? It's likely to happen."

"Yes, it has been shown that you can get culture-positives/false-positives from exactly the scenario that you are saying there," said Professor Gerrit Coetzee of South Africa's National Health Laboratory Services. "Yes, the test is DNA-based PCR, so it will give you dead bacilli – and that's of course the reason why you can't use it as a 2-month test [to monitor response to treatment] because the DNA lingers for quite a while longer."

But another member of the audience pointed out: "Because of the setting we're collecting the samples in, patients might inhale organisms from other patients even though they are not really infected; and a sensitive test could then pick that up."

Indeed, if one thinks about it, there would be an increased risk of this scenario during household contact tracing, shortly before sputum samples are expectorated.

"You will get more false-positive cultures from your kind of scenario than you would of the GeneXpert. But I think that that is negligible," said Prof Coetzee. "It is not just one gene, you need about 150 to 300 colony forming units per ml of sputum before it will turn positive – that's not one TB bacilli, that is probably a couple of thousand of them that goes into this blender."

That has probably been the NHLS's experience for the vast majority of cases that present for care (generally with symptoms) but active case finding and household screening, as discussed in this and the previous HATIP, would involve more transitional or 'borderline cases' where transmission events could be occurring. The dynamics of TB susceptibility and transmission can be puzzling when several people may all have close contact with a person with highly infectious TB and yet have very different responses. Some simply don't become infected, either because they didn't inhale any infectious particles (which seems unlikely in cases with very close contact), or any infectious TB particles they inhaled didn't land on suitable tissue for infection. It's possible that some may get minor MTB infections that are quickly cleared away without even becoming latent TB. The majority of people who get latent TB never develop reactivation TB, people living with HIV being the unfortunate exception to this rule.

In homes visited for contact tracing, by definition, there is at least one person who has been diagnosed with TB (who would now be on treatment) and there is a genuine risk of another person or two in the household with undiagnosed active disease. But other members of the household may be harder to characterise. As already noted, some may have just recently inhaled infectious droplet bacilli and others may be anywhere along the spectrum of responses to TB exposure described above. In a setting with a high frequency of transmission, such as much of South Africa, people with low but detectable levels of MTB may be identified more often in household contact tracing and active case finding than in routine clinical practice. The nature of what is being measured by the laboratory assay could have important clinical implications for patients.

Most of what is known about the natural history of *MTB* infections, and the clinical course and management of the disease came from a time when diagnostic tools were much less sensitive – and it is not clear that a positive result with a very sensitive test really means the same thing clinically as a positive AFB smear or solid culture.

A liquid culture system such as MGIT is the most sensitive test when it comes to live TB bacilli, as it can identify as few as 10-100 colony-forming units (cfu) in a specimen. But if there has been ongoing TB transmission in the house, there's a good chance the home visit could come at a time when it can capture cases of primary TB infection – MTB can be cultured in children with recent primary TB infection who have no symptoms or signs indicative of disease.<sup>5, 6</sup>

Meanwhile, according to studies cited in the WHO recommendation for the lab test, the assay has analytic sensitivity down to five genome copies of purified DNA, and 131 cfu/ml of *M. tuberculosis* spiked into sputum – so more or less similar to liquid culture. If specimens are collected at the right time and at the right place, there would seem to be a chance that the test could be amplifying genes from dead, inactivated TB mycobacteria months after primary infection. Such cases wouldn't be confirmed by culture, but if screening happens at the right time and place, this seems destined to occur in high burden settings.

Professor Coetzee believes the most common reason for a positive result on GeneXpert and negative result on culture is contamination in the lab.

"You are going to get the isolated case where you have a positive GeneXpert, and it doesn't grow – the usual thing is it's going to be contaminated inside the laboratory. When we get there we will make a plan – we take a second specimen," Prof Coetzee said.

As HATIP has noted in previous issues, Professor Ben Marais (who has now moved from South Africa to the University of Sydney), believes that simply because low levels of MTB can be detected by the newer lab tests, it may be a mistake to conclude that these cases who are smear-negative on microscopy, without major signs and symptoms of disease, are all incipient reactivation TB.

"You are asking a key question that the adult TB world have chosen to disregard in the race to improve case detection," Prof. Marais told HATIP. "The fundamental question remains though – what is it that we are measuring? From the natural history of disease studies in children we know that transient MTB excretion following primary infection is a common entity and that the majority of these children never progress to active disease."

"It is reasonable to expect that transient organism excretion may occur following recent reinfection events as well and in certain TB endemic areas in SA, infection/reinfection may occur multiple times each year, which implies that at any one time there may be many people out there - transiently excreting minute amounts of *M.tb*

(that can be detected by very sensitive tests such as culture or PCR) – but likely on their way towards self-containment," he added.

According to Prof. Marais positive culture/GeneXpert in an asymptomatic individual could mean:

- Subclinical or incipient disease (likely in severely immune compromised; may result from primary or re-infection or re-activation)
- Transient excretion following recent primary infection or re-infection (more likely in a high-burden setting; low risk of disease progression in immune competent individuals)
- Transient excretion as a result of subclinical reactivation (more likely in a low-burden setting; not sure how to quantify risk – high in immune compromised as above)

"This presents a clinical dilemma and it would be important to differentiate transient organism excretion, after primary infection or reinfection, from active disease," he wrote in one paper.<sup>7</sup>

## Clinical management in asymptomatic cases

Until such issues are sorted out, healthcare providers may have to stay on their toes to manage such cases clinically.

"Essentially we've been treating patients clinically, even with negative smear results and negative culture results," said Dr Jonny Peter of the University of Cape Town's Lung Institute during the session at the South African AIDS Conference. "I think there are going to be those situations that arise, and I think it's going to necessitate using clinical judgement on an individual patient basis."

"Yes we do know that asymptomatic or subclinical TB exists, but we have a poor grasp of how `stable` this state is (seems like not in most people). and how important it is with respect to transmission – given that coughing is an important part of spreading infection," Dr Liz Corbett of the London School of Hygiene and Tropical Medicine told HATIP.

She added that in research she's been involved in, follow-up of asymptomatic culture positive participants in prevalence surveys (for the research goal of `case-ascertainment` as well as for making sure that disease is treated) has shown that cases who remained culture positive had developed symptoms by the time a culture result was available – though she acknowledges this could be different with GeneXpert.

"Sometimes we found that there were symptoms originally to which the person didn't want to own up [*since TB is highly stigmatised*]. Sometimes the person was now culture-negative and completely fine with no evidence of TB disease over several months of follow-up – perhaps the `natural history` of recent TB infection that Ben has reviewed. But most such instances in HIV-infected people progress to obvious symptomatic disease within a few weeks," she added.

HIV-positive individuals are at a greater risk of progression even with primary disease, though this probably depends on the state of immune suppression and viral load. Even if a case is primary or reinfection TB, as Dr Lawn has pointed out in another recent study, asymptomatic HIV-positive people with evidence of TB (on culture) do tend to progress to symptomatic disease within months to a year.<sup>8</sup> In another cohort of people with somewhat less advanced HIV disease, Oni et al reported that 56% of asymptomatic culture-positive TB cases that were followed up developed symptoms three days to two months later.

"I have no problem treating such culture-positive patients (albeit in the context of a reliable lab) and I have not seen a convincing false-positive GeneXpert TB diagnosis and so would readily treat all



GeneXpert-positive patients too as it replaces culture in South Africa,” Dr Steve Lawn told HATIP.

“Clearly we should improve case detection, and a positive culture in a highly immune compromised person is a significant result that probably justifies TB treatment, irrespective of the presence of clinical symptoms or signs of disease since this is justifiable from a risk/benefit perspective,” Prof. Marais told HATIP.

The risk of progression also may differ between infection and reinfection in HIV negative people — it has been theorised that multiple reinfection is what snaps the disease out of latency.<sup>9</sup> This means that if people live in settings where the risk of exposure is high, they probably are at greater risk of progression, even if HIV-negative (which could be part of what contributes to the high burden of TB in parts of South Africa).

Observation and continued screening may be called for in some of the smear-negative cases — but treatment would be warranted in anyone who becomes smear-positive.

“We published some work from Zimbabwe about 10 years ago which suggested that most people progressed rapidly to treatment-seeking for symptomatic disease once they were smear-positive, but a small percentage do remain smear positive and undiagnosed for very long periods even when access to culture-based diagnosis was right on their doorstep,” Dr Corbett told HATIP.

“If either resolution to latent TB or rapid progression to symptomatic state is the general rule for almost all asymptomatic cases, then repeated symptom screening (as in HIV clinics) will pick the people who are progressing the next time they attend, and, dare I say it, isoniazid preventive therapy will sort out the newly latent ones!” she added.

One of the other reasons we need to learn whether these cases are reactivation, versus transient primary infection or reinfection, is that researchers could be reaching inaccurate conclusions about what should be the gold standard for TB diagnosis, and health departments will be poorly guided when choosing the optimal diagnostic algorithm for their programme, or perhaps cause them to adopt an unnecessarily expensive strategy.

Of course, it would be difficult to suggest conducting a study where one would randomise smear-negative cases without symptoms of TB to either immediate treatment or observation (in order to see whether and when the participants progress to symptoms and possibly smear-positivity). This could only be done in people without HIV (at first) in order to determine what percentage of these cases are reactivated TB. It may be considered unethical, but one could make the case that HIV-negative people could otherwise be at great risk of overtreatment, because if these are indeed recent transient infections — which will become latent, very few of them are actually at risk of progression (the lifetime risk of latent TB becoming active TB in a person without HIV is only 10%).

Another approach that wouldn't answer the question definitively, but might shed some light on the subject, would be to conduct a study comparing rates of positive GeneXpert, results of negative culture (using solid media) in TB suspects from the same setting but derived from different case finding approaches (household contact screening, HIV ICF, or passive case finding). If the group with the highest transmission rates also has a significantly higher rate of positive GeneXpert/negative culture results, it would suggest that the test could be picking up cases of recent infection.

## Despite the unanswered questions, active case finding is the priority

Given the vast improvement liquid culture and GeneXpert represent over earlier TB diagnostics, it may sound as if we are looking for faults and problems. This is not about failures of the tests so much as about our knowledge of how to use them most effectively and efficiently. If liquid culture or GeneXpert tests are picking up a substantial number of cases that are not reactivation TB, it has a direct bearing on how other studies, such as those conducted by Dr Shapiro and Dr Cavanaugh, should be interpreted — and that would include the lower than expected negative predictive values for the Kenyan and WHO 4 TB symptom screens.

Frankly, to use preliminary results to broadcast to National Health programmes in resource-limited settings that 1) TB symptom screens are inadequate, and that 2) the ONLY way to detect TB cases responsibly is through the use of expensive diagnostics that few can currently afford, seems at the very least to be poorly considered since it will discourage programmes from implementing what they can do to save people's lives.

“Just at the time when we have determined a pragmatic approach to exclude active TB, we seem to be willing to “kill” this approach by highlighting the fact that it fails to find “all” TB cases (even the original Getahun analysis did not promise a 100% NPV),” Dr Annelies Van Rie told HATIP. “From a public health perspective, we need to define the risk/benefit ratio of not implementing ICF/IPT using the 4-symptom screen versus implementing ICF and IPT in asymptomatic HIV-infected individuals and consequently inadvertently placing some people with detectable replicating bacteria on IPT. Unfortunately, we do not yet have the population-based cohort studies to address this issue.”

“A lot more critical thinking is required now that sensitive detection tools are rolled out and used for active case finding. In principle I would caution against “active case finding” amongst completely asymptomatic individuals as we do in children — where inclusion of asymptomatic kids with “culture confirmed TB” has complicated interpretation of vaccine protective effect and potentially derailed vaccine development progress, unless this is reserved for severely immune compromised patients,” Prof. Marais told HATIP.

“Another thing that is rarely discussed is how frequently Xpert/culture-based screening of “asymptomatic” individuals should be performed. Should this be done annually in TB endemic settings where risk of infection is high? With symptom-based screening the guidance would be to screen at every single clinic visit, with further investigation as clinically indicated e.g. with high risk features or any persistent symptoms on clinical follow-up,” he concluded.

“From the resource-poor setting of a country like Malawi, I could never see the resources (human, material and financial) being enough to screen for TB in HIV-infected asymptomatic individuals — be this screening with smears, GeneXpert, urine LAM or culture. To do this equitably and expansively in HIV-infected asymptomatic persons is just too big a job, so the question of finding significant amounts of TB in asymptomatic persons for me is really a non-issue in the real world of poor countries,” Professor Anthony Harries of the Union told HATIP.

Dr Corbett agreed: “There may be a few parts of the world (such as South Africa) where the risk or prevalence of subclinical disease is high enough to make screening of asymptomatics worthwhile, but



in most parts of Africa and elsewhere globally the costs of detecting this type of disease will blow a huge hole in the NTP budget for little gain and at a time when the Global Fund is financially shaky. Also what about culture-negative disease (which will also tend to be Gene Xpert negative)? This could be up to 20% of adult cases and higher for kids.

“We have to focus on identifying TB in PLHIV with symptoms and investigate those with symptoms with the technology the country has decided it can afford,” Professor Harries told HATIP. “Smear and CXR are inadequate, and if GeneXpert could be made more point of care and cheaper this would be a major advance.”

“We do active TB case finding so we can identify TB patients so we can treat them so we can cure them. The bottom line for me is cure, and I think we need to seriously start linking the outputs of active TB case finding with treatment and treatment success. From the limited literature on this, I think we do not do so well. A significant proportion of those TB patients detected by active case finding never start treatment! And this brings me back full circle to the asymptomatic patient – how well do these culture-positive asymptomatic persons do in terms of compliance and adherence with 6 months of TB treatment and do we obtain good cure rates?” concluded Prof. Harries.

Indeed, this issue came up during the question and answer discussion during the Active Case Finding session in Lille. Some clinicians noted they were having trouble convincing many of the culture-positive asymptomatic cases to present for care.

“Even if you do detect them, the very small number of people who are really in a stable asymptomatic culture-positive state are not all that straightforward to manage once you do make the diagnosis,” Dr Corbett acknowledged. “Understandably they are reluctant to accept a diagnosis of TB, and need confirmatory tests and the time to talk them into being convinced that they do indeed have TB. This is beyond what can be expected from basic clinic staff in most African settings—and at worst can go completely the wrong way and generate a lot of anger and being threatened with public health legislation.”

“So for both public health and individual benefit of early diagnosis, I would also argue that we **focus first on implementing TB symptom screening well**. Where funds permit and prevalence rates of asymptomatic TB are high, then there can be more comprehensive TB screening regardless of symptoms, but this will not be a realistic goal in most HIV clinics. And in that case there needs to be some special consideration of how you confirm the need for TB treatment in asymptomatic people—bearing in mind that screening is just that, and that the guidelines for managing screening programmes stress very strongly that a positive screening test should not be assumed to be confirmatory, no matter how good the diagnostics,” Dr Corbett concluded.

## Introducing new tools to support diagnosis

Nevertheless, even if the emphasis should be first to do symptom screening well, this will still result in huge numbers of people (those who screen positive) who will then require access to these diagnostic tools anyway.

“If national HIV programmes do adopt and operationalise WHO guidelines [for ICF], clearly there will be major implications for the laboratory and radiology workload as patients who screen positive are referred for evaluation,” said Dr Desai.

He noted that in Getahun et al’s ICF meta-analysis (upon which the 4 symptom screen is based), almost *half* of the 8100 people living with HIV included had screened positive for TB symptoms. More aggressive HIV counselling and testing programmes mean that CD4 cell counts should be somewhat higher than in the cohorts

analysed in Getahun et al, so there may be a lower proportion of TB suspects (but probably a higher absolute number).

Nevertheless, it is clear that initially at least, there will be an increase in workload for any programme. Consequently, programme managers will need to develop plans for laboratory strengthening, including training, hiring staff, equipment, commodities, laboratory information systems for recording and reporting results.

More on how programmes might do that and what role new tools might play (whether they are affordable and which algorithms might be cost effective where) will be discussed in the next HATIP in this series.

In the meantime, the discussion from which many of the expert comments came is open on [HATIP’s blog](#), and we invite other researchers, clinicians, public health specialists and activists with views on the matter to join the discussion there.

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## about HATiP

A regular electronic newsletter for health care workers and community-based organisations on HIV treatment in resource-limited settings.

The newsletter is edited by Theo Smart (Cape Town) and Keith Alcorn, NAM's Senior Editor (London).

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