

# An eXpert AFB Smear?

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(See the Brief Report by Theron et al, on pages 384–8.)

The development, evaluation, and World Health Organization (WHO) endorsement of the Xpert MTB/RIF automated real-time polymerase chain reaction (PCR) assay has generated considerable excitement as a revolutionary diagnostic test for tuberculosis. However, one of its limitations is the inability to determine which patients with pulmonary tuberculosis have sputum positive for acid-fast bacilli (AFB) on microscopy, the current laboratory indicator of infectiousness [1]. The AFB smear is currently used to guide infection control practices and household contact investigations across the globe, and to monitor response to treatment. How the Xpert MTB/RIF assay can fill these roles is unknown. Given this uncertainty, Theron et al [2] evaluated the relationship between quantitative cycle-threshold ( $C_T$ ) data generated by this system and conventional AFB sputum smear data from tuberculosis suspects in Cape Town, South Africa, a setting with a high rate of tuberculosis–human

immunodeficiency virus (HIV) coinfection. In this issue of *Clinical Infectious Diseases*, they report their findings and conclude that the  $C_T$  is a moderately good test for ruling out smear positivity of sputa specimens but a poor test for confirming or ruling in smear positivity.

The Xpert MTB/RIF assay uses real-time PCR technology to detect both the presence of *Mycobacterium tuberculosis* DNA and rifampin resistance based on presence of the *rpoB* gene and its mutations. A sputum specimen is mixed with a sample reagent and homogenized before transfer to a cartridge that is then placed into the closed system. The  $C_T$  refers to the cycle number at which the fluorescent signal from the molecular beacon probes becomes detectable above the background [3]. An excellent review with more detail on the development, evaluation, and implementation of this new diagnostic test has recently been published [4].

The study reported by Theron et al [2] was conducted by a well-respected, experienced research team in Cape Town, South Africa, a setting beset by a devastating epidemic of tuberculosis complicated by high rates of HIV coinfection and multidrug-resistant tuberculosis (MDR-TB). To make matters worse, South Africa is now the epicenter of extensively drug-resistant tuberculosis, with well-documented nosocomial transmission to both patients and healthcare workers [5, 6]. Given the strong recommendation from the WHO that the Xpert

MTB/RIF be used as the initial diagnostic test in individuals suspected of MDR-TB or HIV-associated tuberculosis [7], the setting for this study could not be more appropriate. Much of the literature on the Xpert MTB/RIF to date has been from the developers of the assay, so this article from an independent group is welcome, even though they received funding from the Foundation for Innovative New Diagnostics, one of the developers. The rationale for using the  $C_T$  as a surrogate for bacillary concentration is supported by the log-linear relationship observed between the  $C_T$  and quantitative culture from the original developers [8] and by the significant correlation between the  $C_T$  and days to positive in liquid culture reported by this group in the parent study of this report [9].

The study from Theron et al [2] is similar to another recent report from Blakemore et al [10], the original developers of the assay. Both are follow-up studies using cohorts from well-designed parent studies on the performance characteristics of the assay for diagnosing pulmonary tuberculosis. In the Blakemore et al study [10], there were 741 tuberculosis suspects from 5 sites, 2 of which were in South Africa and were the only sites with high rates of HIV coinfection [11]. Blakemore et al [10] included only samples from patients with culture confirmed tuberculosis, whereas Theron et al [2] included all 496 of the original tuberculosis suspects. Both

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studies used pretreatment specimens. Theron et al [2] used different specimens for fluorescent microscopy on concentrated smears and Xpert MTB/RIF, whereas Blakemore et al [10] split the sputa specimens between Xpert assays and Ziehl-Neelsen staining for microscopy of both direct smears and centrifugates. Blakemore et al [10] found that the correlation between the  $C_T$  and the AFB sputum smear and liquid culture time to positive was strongest for assays that had internal control (to identify PCR inhibition)  $C_T$  values  $<34$ , although there was considerable variation among sputum samples within the same AFB grade. Theron et al [2] did not mention use of the internal control data. However, they assessed the use of a clinical prediction score to augment the  $C_T$ , although it did not appear to be clinically helpful. Theron et al [2] used more complicated statistical analyses to assess cut-points to rule in and rule out smear positivity as well as the Youden index, a measure of overall diagnostic effectiveness balancing sensitivity and specificity [12]. Blakemore et al [10] used more straightforward Spearman correlations. As a simple comparison, Theron et al [2] found that a cutoff of 27.1 for the Youden index has a sensitivity of 82.3% and a specificity of 79.4%, and Blakemore et al [10] found that a cutoff of 27.7 was 98% sensitive and 48% specific for smear-positive status. The differences in the sensitivities and specificities are likely explained by the variability in patient populations and methods discussed above. The similarity of these cut-points is striking given these differences. Additional analyses from Theron et al [2] suggest that smear positivity could be ruled out with a higher cut-point of 31.8 with a sensitivity of 95.8% and a specificity of 47.1%.

In the multivariate model used to derive the clinical prediction score, Theron et al [2] found that HIV was not a significant predictor of smear status, probably due to the  $C_T$  being a better marker

of bacterial load. This is interesting in light of the finding from their parent study that HIV coinfection was associated with a trend toward lower sensitivity ( $P = .09$ ) and significantly reduced negative predictive value ( $P = .001$ ). They also noted a lower sensitivity (55%) among smear-negative cases. Although the Xpert MTB/RIF has been found to have significantly increased sensitivity over AFB smears in multiple studies, it is still obviously dependent upon the bacillary concentration in the sample. This is a significant limitation especially relevant to its use for patients infected with HIV.

A limitation common to both studies is the reliance on the AFB sputum smear as the gold standard marker of infectiousness. For example, approximately 30% of sputum smear-positive patients have been found to be infectious using different methods, and there is a 3-log range of infectiousness among them [13]. In addition, approximately 15% of tuberculosis transmission has been associated with smear-negative cases [13]. An additional problem in using the AFB smear as the gold standard is the variability among AFB smears. Do we use direct smears, as were used in the early large studies of transmission? Or do we use fluorescent microscopy, with or without centrifugation? Unfortunately, the AFB sputum smear can really be no more than a “tin standard” for infectiousness, and it should be considered only a risk factor for infectiousness rather than the universal standard [14].

One of the paradigms that I suggest we challenge is the assumption that a diagnostic test of disease is the same as a diagnostic test of infectiousness. Tuberculosis likely follows the “20/80 rule” found true for many infectious diseases—that approximately 20% of cases transmit approximately 80% of the disease [15]. The reasons for this variability are poorly understood. As suggested by Theron et al [2], to best understand the  $C_T$  as a potential marker of infectiousness, we will need prospective

transmission-based studies (eg, among household contacts and/or in animal models). My personal bias is that such studies should also include assays of aerosols, because we have known for decades that tuberculosis is transmitted by aerosols and not by sputum.

So should we now retire the AFB sputum smear? I think not. Both of these excellent studies suggest that the  $C_T$  can be used with appropriate cutoffs as a surrogate for the AFB smear and as a marker of bacillary load in pretreatment cases, albeit imperfectly. However, once patients are on treatment, the use of the  $C_T$  for removal of isolation or discharge to high-risk environments is unknown. Similarly, its use as a marker of treatment response has not been studied. The  $C_T$  might be thought of as an excellent quantitative smear, in that both tests measure total viable and nonviable bacilli. I suspect that the  $C_T$  may change during treatment as does the AFB smear, but to my knowledge such studies have not been done or published. There is a need for a large number of operational and implementation studies of the Xpert MTB/RIF assay, and it will be important to continue to compare this assay to the AFB smear in those studies. The Xpert MTB/RIF assay has excellent performance characteristics as a rapid tuberculosis diagnostic tool. How it will integrate into healthcare systems is likely to vary with resources and local epidemiology, and it will be critically important to resolve issues of cost, durability, maintenance, and other factors before bidding final farewell to the AFB smear [4].

## Notes

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Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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