the vasculature more easily. AAV and lentiviral receptors and intracellular processing also differ from adenovirus, further complicating the picture. Available data on AAV suggest that vasodilation, vascular permeability enhancement, and avoidance of inhibitors to infection are still relevant variables. Greenberg and colleagues[4] suggest that the ratio of infectious to total particles should also be a concern, although further investigation is needed to more precisely define this effect.

Where do we go from here? The present strategy of piecemeal methods development is insufficient. A paucity of funding opportunities and the attitudes of grant reviewers for methods development proposals are obstacles to progress. A methods-centric grant has essentially no possibility of funding, and a disease-focused or therapy development grant that admits to issues with delivery methods is equally impaired. In 2003, the US National Institute of Biomedical Imaging and Bioengineering funded several investigators to develop gene delivery methods. Out of that funding programme came many of the advancements in cardiac gene delivery mentioned in this Comment.[11,12,16] I am not aware of any similar funding opportunity in the USA or elsewhere. If we want cardiac gene therapy to work, we need to renew this focus on methods development with an ultimate goal of gene delivery to all myocytes in the cardiac ventricles. When delivery methods are robust and reliable, clinical successes are likely to follow.

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I have five issued US patents (6376471, 6855701, 7256182, 6992070, 7034008) and one pending US patent application (20150183842) within the general field of cardiac gene delivery.

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A breakthrough urine-based diagnostic test for HIV-associated tuberculosis

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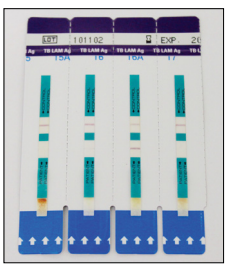
See **Editorial** page 1134
See **Articles** page 1187

At the 44th World Health Assembly in 1991, the burgeoning, and now modern day, tuberculosis pandemic was brought to the world's attention,[1] reporting that 6 million new tuberculosis cases and 3 million associated deaths were occurring worldwide each year. The global HIV pandemic was recognised as a key factor fuelling the deterioration in tuberculosis control, and such were the grim portents of the emerging tuberculosis and HIV co-epidemic in sub-Saharan Africa that, in 1991, three London academics posed the pointed question, "Is Africa lost?"[2] Now, almost a quarter of a century after tuberculosis was declared "a global emergency", roughly 9·6 million new cases of tuberculosis and 1·5 million deaths (0·4 million deaths in HIV-positive individuals) still occur annually.[3]



Andrew D Kerkhoff

Determine TB-LAM test strips

Tuberculosis remains the leading cause of AIDS-related deaths among adult inpatients in resource-limited settings. In nine post-mortem studies done in Africa, between a third and two-thirds of adult deaths related to HIV or AIDS were due to tuberculosis.[4] That almost half of this tuberculosis burden remained undiagnosed and untreated before death is a shameful indictment on our approaches to tuberculosis diagnosis for people living with HIV or AIDS, and shows the woeful inadequacy of the diagnostic methods at our disposal.

Emergence of the Xpert MTB/RIF rapid molecular assay and its subsequent WHO endorsement in 2010 seemingly transformed the tuberculosis-diagnostics landscape. Xpert provided diagnostic accuracy that was far superior to that of the existing sputum-smear microscopy that national tuberculosis programmes worldwide had almost exclusively relied upon. In view of its excellent diagnostic accuracy and ability to rapidly detect genotypic rifampicin resistance,[5] Xpert MTB/RIF was dubbed a potential game-changer. However, findings from subsequent assessments of this assay in randomised controlled trials[6,7] did not match the hyperbole, and no studies to date have shown that its implementation has an effect on mortality; instead, glaring weaknesses in the health systems in which Xpert was deployed became apparent.

Against this historical backdrop, the findings of the study reported by Jonny Peter and colleagues[8] in *The Lancet* are remarkable. They report the results from their randomised trial in four countries in sub-Saharan Africa of the effect of a rapid, point-of-care assay for the diagnosis of HIV-associated tuberculosis on all-cause mortality.[8] 2659 HIV-positive patients were randomly assigned to a tuberculosis diagnostics arm; 1336 individuals to diagnosis with lipoarabinomannan testing (LAM) in addition to routine diagnostics, and 1323 individuals to diagnosis with routine diagnostics alone. The investigators showed that among HIV-positive adults needing hospital admission, use of the urine-based, point-of-care LAM assay in combination with standard of care tuberculosis investigations was associated with a relative risk reduction of 17% (95% CI 4–28) in 8-week all-cause mortality (with a risk ratio adjusted for country of 0·83 [95% CI 0·73–0·96]), and a potentially even greater reduction among more severely immunocompromised patients (hazard ratio for LAM adjusted for country for those with CD4 cell count ≤50 per µL 0·71 [95% CI 0·56–0·90]). This is the first randomised trial to show a mortality reduction resulting from implementation of a new tuberculosis diagnostic assay.

For many years, the development of truly point-of-care diagnostic assays has been foremost on the tuberculosis research agenda. An apparent breakthrough came with the development of the 25-min lateral-flow antigen assay, Determine TB-LAM,[9] which detects lipoarabinomannan of the mycobacterial cell wall in urine.[10] The assay requires neither laboratory infrastructure, nor equipment and costs far less per test (<US$3) than the Xpert MTB/RIF assay (around $10). The LAM test has moderate overall sensitivity but is most sensitive in ill patients with the lowest CD4 cell counts, and has very high specificity.[9,10] Detection of lipoarabinomannan in urine is indicative of haematogenously disseminated tuberculosis with renal involvement in very ill patients with HIV.[11] Thus, the urine-LAM lateral-flow assay is perfectly tailored to diagnose HIV-associated tuberculosis in individuals with the highest mortality risk, accounting for why, in Peter and colleagues' randomised trial,[8] its implementation was associated with such a marked reduction in mortality—especially in patients with the lowest CD4 cell counts.

Evidence about the diagnostic accuracy of the urine-LAM assay was synthesised in a Cochrane review[12] and assessed by a WHO expert panel in June, 2015, alongside data from the trial by Peter and colleagues.[8] Their data formed an important final piece of evidence to underpin WHO's recommendations for use of this assay.[13] As of November, 2015, WHO recommends urine-LAM testing for HIV-positive hospital inpatients with signs and symptoms of tuberculosis, who have a CD4 cell count of 100 cells per µL or fewer, and also HIV-positive inpatients who are seriously ill, irrespective of their CD4 cell count.

Peter and colleagues[8] report on the diagnostic accuracy of urine-LAM testing even though the study was not primarily designed a priori to rigorously assess this.[8] If their finding that urine-LAM specificity was less than 90% is taken at face value, these data would raise serious concerns about the potential of the assay to generate large numbers of false-positive diagnoses. Suboptimum specificity has long been cited as a concern with this assay[10] (although studies suggesting this are almost invariably derived from studies with suboptimum design to rigorously assess diagnostic accuracy). In Peter and colleagues' study, the reference standard for tuberculosis diagnosis against which the diagnostic accuracy of the LAM test was assessed was often only one sputum

culture or Xpert test, both of which represent insufficiently robust reference standards. In a similar inpatient study population in Cape Town, South Africa,[14] our results showed that the specificity of the same urine-LAM lateral-flow assay exceeded 99% when compared with a rigorous microbiological reference standard that incorporated sampling of sputum, blood, and urine, for which we did a mean of 5·6 reference standard tests per patient.

In summary, Peters and colleagues report findings from their landmark trial that provide key evidence showing that urine-LAM testing is an effective means of rapid, low-cost, ante-mortem diagnosis for the large burden of HIV-associated tuberculosis. This burden for the past 25 years has only been brought to light by a long series of post-mortem studies.[4] With the recent backing of WHO recommendations, we strongly advocate that the Determine TB-LAM point-of-care assay should be implemented by national tuberculosis programmes in sub-Saharan Africa to reduce AIDS-related inpatient deaths.

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Why are people living with HIV still dying of tuberculosis?

See **Editorial** page 1134
See **Articles** page 1198

Tuberculosis and HIV have been seen as intertwined since the earliest report of AIDS more than 30 years ago.[1] Despite the remarkable success of the expansion of access to antiretroviral therapy, deaths due to HIV-related tuberculosis remain common. WHO estimated the number of such deaths to be 0·4 million in 2014.[2] This number is not straightforward to estimate. The more clinicians test for tuberculosis in patients with advanced HIV, the more patients with tuberculosis they find.[3] Yet the introduction of more sensitive diagnostic tools has not reduced mortality.[4] For years pathologists have highlighted that many patients dying with HIV infection do so either from or with tuberculosis, and that the diagnosis of tuberculosis had often not been made ante mortem.[5,6] Epidemiologists show that in adults found in random population samples from