stem cells is the standard by which their pluripo-
tent potential is evaluated.\textsuperscript{1-3} We believe that the
intended application of human induced pluripo-
tent stem-cell lines should determine the evalua-
tion method. The issue of pluripotency becomes
important for studies of the functional mecha-
nism of reprogramming. However, a reproduc-
ible and rapid method to determine the quality of
newly established human pluripotent stem cells
is urgently required. Perhaps an epigenetic and
gene-expression signature that selectively defines
fully reprogrammed pluripotent stem cells can be
identified, since the variation of teratoma results
brings up the question of this assay’s value as a
standard for proving pluripotency of human
stem cells.\textsuperscript{4}

We tested our lines for the reactivation of en-
dogenous pluripotency genes, the silencing of ret-
roviral transgenes, and the capacity to form the
three germ layers. In close collaboration with the
pathology department, we are in the process of
correlating the results of teratoma assays with the
ability to generate functional cardiac myocytes.
Although induced cardiomyocytes may replace
pluripotent stem cells for disease modeling, this
technique needs to be reproducibly established in
human cells.

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Rapid Molecular Detection of Tuberculosis

\textbf{TO THE EDITOR:} Boehme et al. (Sept. 9 issue)\textsuperscript{1}
report encouraging results on the use of an auto-
mated molecular test for \textit{Mycobacterium tuberculosis}
and resistance to rifampin (Xpert MTB/RIF). How-
ever, the population of patients with clinical tu-
berculosis who have negative cultures still poses a
problem of interpretation, which was not dis-
cussed in the article. Among study patients whose
samples were culture-negative but who had symp-
toms of tuberculosis, 29.3% had positive results
on the automated test; these patients made up 4.3% of the total number of automated test–pos-
itive patients.

In such patients, tuberculosis that was detect-
ed by the automated test may have had a false
negative culture because of low bacillary load or
overgrowth, but the possibility of false positiv-
ity cannot be excluded. Furthermore, 23 patients
with nontuberculous mycobacteria in culture were
excluded from the analysis. In our site in Tanzania
and in other African locations, nontuberculous
mycobacteria are frequently found in culture,\textsuperscript{2,3}
so the capability of the automated test to dis-
criminate between tuberculosis and nontubercu-
losous mycobacteria, for which preliminary results
have been encouraging,\textsuperscript{4} would be of great inter-
est. More effort should be made in future studies
to elaborate on these two groups, thus clearing an
uncertainty regarding the performance of the
automated test.

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terium tuberculosis and rifampin resistance by use of on-demand,
TO THE EDITOR: Although resistance to rifampin and isoniazid usually occurs concomitantly, rifampin monoresistance is known and well documented in certain populations.1-3 When the automated test detects rifampin resistance, clinicians would have to consider further diagnostic and treatment options. First, would the strain be assumed to have multidrug resistance, given the high probability of concomitant resistance to isoniazid? In that event, treatment options would probably include the remaining first-line agents (ethambutol and pyrazinamide) plus a fluoroquinolone and an injectable antituberculosis agent.4 Clinicians may still prescribe isoniazid until resistance to the drug is excluded. Further testing with the use of either a traditional assay or the Genotype MTBDRplus assay would still be needed. Nonetheless, at least one or more second-line agents would be initiated early in the treatment of the strain of tuberculosis with at least partial resistance. However, patients with rifampin mono-resistance may be unnecessarily exposed to the untoward effects of injectable agents for tuberculosis until the complete drug-susceptibility pattern is obtained. Additional detection of isoniazid resistance would substantially enhance the utility of this test.

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No potential conflict of interest relevant to this letter was reported.


TO THE EDITOR: Boehme et al. report that an automated test for tuberculosis had a sensitivity of 97.6% and a specificity of 98.1% for the correct identification of patients with rifampin-resistant tuberculosis. Even though the test greatly advances the direct detection of M. tuberculosis, correct identification of rifampin-sensitive strains is essential, since the false diagnosis of multidrug-resistant tuberculosis is deleterious for patients.1

We have identified a patient with rifampin-sensitive tuberculosis for whom automated testing of bronchoalveolar lavage falsely showed rifampin resistance. The M. tuberculosis bacterial load detected by the automated test was low. The curve pattern generated in the automated test resulted in the interpretation “rifampin resistance detected.” In contrast, rpoB sequencing,2,3 line-probe testing (AID Diagnostika), repeated culture, and phenotypic drug-susceptibility testing revealed the presence of fully susceptible M. tuberculosis. In this patient, short-course therapy resulted in a clinical response.

A major limitation of the design of the automated test is that interpretation relies solely on a decreased level (or the absence) of wild-type beacon hybridization, rather than additional hybridization to mutant probes, which would add to the specificity of the test. Our results indicate that positive results for rifampin resistance on the automated test must be viewed with caution and should be confirmed by phenotypic or additional genotypic methods when possible.

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TO THE EDITOR: The study by Boehme et al., which reports a high degree of accuracy for an automated test for the identification of tuberculosis in adults, could revolutionize the diagnosis of tuberculosis globally. However, in high-burden settings, up to 30% of tuberculosis cases occur in children. The diagnosis in this age group is challenging, especially in the high-risk groups for disease progression and poor outcome, which include children who are under the age of 5 years.
those who are malnourished, or those who have concomitant infection with the human immunodeficiency virus. The presentation of tuberculosis in children is often nonspecific, with few bacilli, and may progress rapidly to severe forms.1 New diagnostic tests with improved accuracy in children with smear-negative and extrapulmonary disease have the potential to substantially improve case management and surveillance. Such tools are urgently needed.

It is thus essential that high-quality studies of new diagnostics that use rigorous and standardized approaches should be conducted in children, despite the lack of a sensitive reference standard for comparison. The game change2 is welcome, but the new strategies should also prioritize tuberculosis research in children.

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TO THE EDITOR: Rapid molecular detection of tuberculosis and isolated rifampin resistance may be cost-effective with some constraints. Such testing does not detect rifampin resistance caused by mutations outside the core region of the rpoB gene, and a single mutation might generate a different resistant phenotype. The presence of mutations within the rpoB locus that are not associated with resistance may influence the annealing properties of the probes. Thus, a substantial number of strains can be classified as resistant on genetic analysis and as sensitive on phenotypic testing.1,2

The efficiency of this nested polymerase-chain-reaction (PCR) assay is ordinarily lowered when a reduced number of acid-fast bacilli (AFB) are encountered in sputum samples. Since the detection limit of such a system is generally 10 mycobacterial genome copies, sputum samples with a score of AFB 1+/rare may contain smaller amounts of required DNA to be amplified.3

The study by Bohme et al. was conducted for 9 months. When used routinely for a long time, PCR assays can have false positive results because of amplicon or chromosomal DNA contamination.4 Therefore, quality control and assurance is difficult in resource-poor countries in which tuberculosis is endemic. The genotyping method probably will not replace conventional phenotypic methods in the near future.

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THE AUTHORS REPLY: Heinrich et al. question the ability of the automated test to differentiate M. tuberculosis from nontuberculous mycobacteria. In a study by Helb et al.,1 the automated test did not significantly cross-react with nontuberculous mycobacteria. Moreover, mixing nontuberculous mycobacteria at 105 colony-forming units (CFU) per milliliter with 200 CFU of M. tuberculosis did not interfere with the detection of either M. tuberculosis or rifampin resistance. The automated test can allow detection of M. tuberculosis despite the colonization of nontuberculous mycobacteria in culture. In our report, 3 of 23 patients who were excluded because of cultures that were positive for nontuberculous mycobacteria had positive results on the automated test. Of these patients, 2 had cultures with mixed nontuberculous mycobacteria and M. tuberculosis. The single patient who was culture-positive for only nontuberculous mycobacteria was tested-positive in all three sputum samples, with typical PCR curves. This patient probably had tuberculosis, with cultures overgrown by a fast-growing nontuberculous mycobacteria.
We agree that the automated test can detect culture-negative tuberculosis, though quantifying this finding would have required a different study design. Specificity in culture-negative patients cannot be determined in those who are treated for tuberculosis on clinical grounds, since microbiologic follow-up is compromised. The high specificity we found for direct testing in more than 600 untreated patients (99.2%) is not surprising, since the test targets a tuberculosis-specific sequence, and analytic studies have not shown cross-reactivity with 89 other pathogens or respiratory commensals. Though heminested, the reaction takes place in a closed cartridge, and there have been no reports of ampiclon contamination.

Bhanot and Mohapatra raise important questions concerning the use of testing for rifampin resistance in treatment decisions. Rifampin resistance is highly predictive of multidrug resistance in most settings. Furthermore, both multidrug-resistant and rifampin-monoresistant strains of tuberculosis are associated with poor treatment outcomes. Thus, rifampin resistance that is detected by the automated test would probably trigger treatment for multidrug resistance as well as expanded drug-susceptibility testing, depending on local epidemiologic factors.

Mohapatra is concerned about relying on mutations in the rpoB core region to detect rifampin resistance. Numerous studies have shown that this region encodes at least 95% of all rifampin-resistant tuberculosis. These studies are further supported by the clinical performance of Genotype MTBDRplus, which targets the same rpoB region as the automated test. Mutations in this region almost always signify rifampin resistance. Recent reports suggest that certain rpoB core mutations identify rifampin resistance that is often missed by liquid-culture methods but is still detected by testing on solid media. We agree that genotypic testing will not replace phenotypic testing, at least in the short term, but will allow more rapid and decentralized detection of drug resistance.

Zbinden and colleagues are correct that although the specificity of the automated test for rifampin resistance was close to 100% in our study, rare false positive results for rifampin resistance have been reported subsequently by users. Such false calls would have special relevance in settings with a low prevalence of multidrug-resistant strains. The selected assay format enables the detection of virtually all rifampin-resistance mutations. To resolve potential false calls, minor modifications are currently being made to the assay, which will further improve its overall accuracy for the detection of rifampin resistance.

We welcome the comments of Hesseling et al. and can only agree that additional studies in children are a priority.

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Therapeutic Hypothermia after Cardiac Arrest

TO THE EDITOR: The midazolam dose of 0.15 mg per kilogram of body weight per hour described in the article by Holzer (Sept. 23 issue) and in other articles on therapeutic hypothermia is very similar to the recommended dose of midazolam used to adapt critically ill patients without neurologic problems to mechanical ventilation. However, this dose may be excessive in comatose survivors of cardiac arrest who are undergoing therapeutic hypothermia; these patients probably