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Appropriateness of using two instead of a single sputum specimen for monitoring treatment of pulmonary tuberculosis

According to the still officially valid procedure,[1] Category I pulmonary TB (PTB) patients should have their intensive phase extended by 1 month if positive at the end of the second month of treatment. By the same token, a PTB patient who becomes smear-positive again at the fifth month of treatment or later is defined as a treatment failure and reclassified as Category II PTB. As outlined in reference 1, two sputum specimens should be collected for treatment monitoring, yet often only a single specimen is being used. As an example, one study indicated that on comparing the yield of three sputum specimens collected by the end of each month of treatment with the virtual situation of assessing the yield of the first specimen, about 10% of positive results would be lost by the end of the second and third month.[2] This difference would, however, be much lower in the later months.

In the frame of a controlled clinical trial (data not shown), we always collected two overnight sputum specimens at the end of the second and fifth months. The maximum time between the first and second sputum specimens was 4 days for the second month and 7 days for the fifth month of treatment, with the majority showing a space of only 1 or 2 days' difference for both monitoring intervals. The sputum specimens were processed and smears prepared and stained according to the conventional auramine method for fluorescence microscopy. The smears were always read by the same person.

Of a total of 785 specimens collected, 52 were Sm+ by the end of the second month: 26 Sm+/Sm+, 13 Sm+/Sm−, and 13 Sm−/Sm+.

By the end of the fifth month, 13 specimens were Sm+: 11 Sm+/Sm+, 0 Sm+/Sm, and 2 Sm−/Sm+.

Thus, by the end of the second month, 13 patients (25%) fell into the category Sm−/Sm+. These persons would not have received an extended intensive phase of chemotherapy if only a single specimen had been evaluated. The importance of collecting two specimens by the end of the fifth month could not be assessed correctly because of very small numbers.

Clearly, under many conditions our system of two overnight specimens is not applicable. However, provided the guidelines as published in reference 1 are still agreed upon as being universally valid (for discussion on this issue see reference 3), then two specimens should clearly be collected for treatment monitoring by the end of the second month.

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[A version in French of this article is available from the Editorial Office in Paris and from the Union website www.iuatld.org]

A microplate indicator-based method for determining the susceptibility of multidrug-resistant *Mycobacterium tuberculosis* to antimicrobial agents

In a recent issue of the *Journal*, Morcillo et al. reported on a microplate indicator-based method for determining the susceptibility of multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) to antimicrobial agents.[1] The test (M-MTT) is based on the reduction of the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to a visible purple formazan for determining minimal inhibitory concentrations (MIC) of several first- and second-line agents acting on MDR-TB. This approach has been used by other researchers to detect rifampicin (RMP) resistance in clinical isolates of *M. tuberculosis* in a very simple tube format.[2] Recently, Abate et al. standardised the MTT assay for direct detection of RMP-resistant *M. tuberculosis* in sputum samples.[3] This direct MTT assay had a sensitivity and specificity comparable to standard indirect susceptibility testing on 7H10 medium, with results obtained within 2 weeks for 98.5% of the samples studied.

We have previously reported the development of a method for detecting MDR-TB based on the reduction of resazurin in a microtiter assay plate.[4] The method was tested on 80 clinical isolates against isoniazid (INH) and RMP, with results obtained after 7 days. The specificity and sensitivity were excellent as compared to the proportion method performed on Löwenstein Jensen medium. The same assay has been standardised for the second-line drugs ethionamide, kanamycin, capreomycin, ofloxacin and PAS, and tested on 150 *M. tuberculosis* isolates.[5] MICs were obtained after 8 days of incubation with very good correlation with the proportion method.

Colorimetric methods based on the reduction of tetrazolium salts such as those described above have demonstrated reliability and simplicity and seem to be good alternatives for rapid detection of MDR-TB and for drug susceptibility testing against other anti-tuberculosis drugs. However, as mentioned by Morcillo et al. in their article, the real usefulness of these tests should be evaluated in clinical trials performed in endemic countries with a high prevalence of resistance to the drugs under evaluation. These trials should also give an idea about the real costs of these rapid tests and the feasibility of their implementation in low-resource countries, where the problem of tuberculosis and drug resistance is more prevalent and where programmes for re-treatment of MDR-TB patients are available. Furthermore, the successful implementation of this type of test directly on sputum samples would be a major contribution to rapid detection of MDR-TB.

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In reply

We agree with Palmino and colleagues, and we would like to emphasise the potential usefulness of the microdilution colorimetric method whose design can easily be adapted to test several drugs and even combinations of drugs.[1,2] The microtiter plate format could also be used to explore the susceptibility of other mycobacteria such as *Mycobacterium avium* complex to a wide spectrum of antibiotics and even new compounds whose capability to kill these bacteria could be explored.[1–3] Furthermore, our experience with testing classical anti-tuberculosis agents suggests that this system can be suitable for clinical practice.[4]

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