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In their recent article, Kristian Ångeby et al. (4) reported a novel test for drug susceptibility testing of *Mycobacterium tuberculosis*. However, it should be clarified that this method was previously published in 1989 (3) by Emil Kalfin, (National Institute for Lung Diseases, Sofia, Bulgaria) and Andreas Engibarov (National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria). It is not clear where and when the test was first developed but it is apparent that Dr. Kalfin experimented with the methodology for some time prior to its publication. Since 1989, this assay has been used routinely by several diagnostic tuberculosis laboratories in Bulgaria, where the method is known as “the test for nitrate reductase activity” or as “the biochemical test” for assessment of drug susceptibility of *M. tuberculosis*.

During the period of 1998 to 2000, we modified and improved the nitrate reductase assay by experimenting with the use of a crystalline nitrate reductase reagent described by Lampe (5) and Warren (6). This crystalline reagent is reported to be less toxic and has a longer shelf life than the Griess reagent. We tested this new version of the assay on 31 clinical isolates of *M. tuberculosis* collected at Iskrez Hospital, Bulgaria, and two reference strains, *M. tuberculosis* INH-R ATCC35822 and H37Rv, using rifampin (40 µg/ml), isoniazid (0.2 µg/ml), and ethambutol (2 µg/ml). The results were compared to those obtained by Canetti's proportion method as recommended by the World Health Organization (1). The tubes used for the nitrate reductase assay were prepared by including the respective drug concentrations and 0.1% potassium nitrate (KNO₃) to the Lowenstein Jensen medium before inspissation. The KNO₃ reduction was assessed at 8 to 10 days by adding a small amount of the crystalline nitrate reductase reagent (5) to the liquid condensed in the bottom of the tubes. The quantity of the reagent added was not critical. We found 100% agreement of the results for the susceptible strains tested. Discordance with the resistant strains was expressed in borderline susceptibility by one of the methods applied.

We found that the main advantages of using the crystalline reagent were the prolonged shelf life (more than 4 years), the ease of reagent preparation, and the simplicity in performing the test. The changes in color observed when using the Griess reagent (2) are at times instantaneous, but with some strains they may be gradual, taking approximately 30 s. When using the crystalline reagent these problems are rarely observed. We believe the nitrate reductase assay has several advantages when compared to other susceptibility tests in that it is rapid, simple, and inexpensive. In addition, the tubes are inoculated with high concentrations of bacteria, enabling a representative sample of the primary isolate to be tested. We concur with Kristian Ångeby et al. (4) that it may be possible to apply the nitrate reductase test directly to microscopy-positive sputa, thus drastically reducing the time needed for detection of drug-resistant *M. tuberculosis*.

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Authors' Reply

We read with great interest the letter by Panaiotov and Kantardjiev referring to our recently published paper (2). Unfortunately we were not aware of the use of this technique in Bulgaria and the recommendation of it by Kalfin and Engibarov in a Bulgarian guideline for microbial diagnosis of mycobacterial infections in 1989 (1).

However, we find it very promising that the nitrate reductase assay has been in routine clinical use for more than a decade in several Bulgarian microbiological laboratories, and it is unfortunate that the extensive experience of this assay in Bulgaria is not available to the scientific community. Nevertheless, we are happy that this technique has been applied much more than we thought.

Even though we have not yet had the time to test it, we believe that the use of crystalline reagents as suggested by Panaiotov and Kantardjiev, leading to a simplified test performance and a prolonged shelf life, will further improve the potential of this easy-to-perform, rapid, and inexpensive susceptibility test. We look forward to seeing the results from Bulgaria—and other settings—in international jour-

nals and welcome scientific collaboration in this field in the future.

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