A fully automated non-radiometric mycobacteria culture system, MB/BacT (Organon Teknika Durham, NC), was recently introduced in Japan and evaluated for its ability to detect mycobacteria in clinical specimens (mostly sputa). A previous study yielded nearly a 40% contamination rate from sputa treated with the standard NALC-NaOH method. This study employed a mucolytic agent (semi-alkaline protease) in which the sputa were processed twice for digestion at twice the standard volume of NALC-NaOH for decontamination. The concentrated sediments were re-suspended in phosphate buffer (pH 6.8), and inoculated into the MB/BacT Process Bottles supplemented with antibiotics (MAS). The bottles were incubated at 37°C and monitored for up to fifty-six days. Recovery of mycobacteria was compared in three different egg-based Ogawa media in addition to a non-selective Middlebrook 7H10 agar. A total of 1,043 clinical specimens have been evaluated to date. Of these, 456 were positive for growth of mycobacteria, of which 432 (94.7%) were positive by MB/BacT. False positive outcomes due to contamination, mainly by P. aeruginosa and Candida spp., were observed in twenty specimens (1.9%). The three Ogawa media could detect only 280 (61.4%) to 345 (75.7%) positives, and Middlebrook 7H10 agar only 419 (91.9%) positives. The time to detection for positive samples of M. tuberculosis by MB/BacT ranged from 2.3 days to 52.3 days with a mean of 17.4 days. It can be concluded that MB/BacT is particularly useful for the isolation of mycobacteria and has a faster time to detect than conventional methods. MB/BacT is a suitable alternative method for the detection of mycobacteria in Japan, where the radiometric Bactec System is not available.