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Efficiency of the TK Culture System in the diagnosis of tuberculosis☆

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Abstract

We have evaluated the efficiency of the TK Rapid Mycobacterial Culture System in isolating mycobacteria from clinical samples and in susceptibility testing. The TK Medium indicates mycobacterial growth by changing its color from red to yellow. During a 1-year period, 16,303 clinical samples were inoculated to TK selective (TK SLC) and Löwenstein–Jensen media (LJ). Mycobacteria were isolated in 2150 (13.04%) samples in at least 1 type of medium. While LJ isolated mycobacteria from 1920 (11.69%) of all samples, TK SLC isolated 2070 (12.63%). Among all positives, the isolation rates for LJ and TK SLC were 89.30% and 96.27%, respectively. Contamination of cultures by other organisms was observed in 878 (5.33%) LJ tubes and in 90 (0.55%) TK SLC tubes. On average, time-to-growth detection was 15.57 days in TK SLC and 25.14 days in LJ. The modes of time-to-growth detection were 12 and 25 days for TK SLC and LJ, respectively. The reliability of antimycobacterial susceptibility testing was checked by 36 *Mycobacterium tuberculosis* strains with known susceptibility patterns which were obtained from the World Health Organization collection and by participating in an external quality control program. All susceptibility results, except for a few borderline-resistant strains, were consistent with the expected susceptibility patterns. The TK Rapid Mycobacterial Culture System is a practical and reliable automated system that shortens the time required for both culture and susceptibility results. All types of TK Media are ready to use, saving time and effort as well as drastically reducing contamination during testing.

Keywords: Mycobacteria; Rapid culture; Tuberculosis; Diagnosis

1. Introduction

Tuberculosis continues to be one of the major health problems around the world. The most important element in tuberculosis control is to identify patients who have tuberculosis bacilli in their sputum and treat them efficiently, before they transmit the disease to healthy individuals. Culture is the gold standard, the most sensitive and specific method for the diagnosis of tuberculosis. Unfortunately, it takes 3 to 6 weeks to detect mycobacterial growth in classical media like Löwenstein-Jensen (LJ) (de Waard and Robledo, 2007; Manterola et al., 1998; Turkkani et al., 2010). Rapid automated culture systems are not used as widely as classical media, mainly because of their high cost, requirement for expensive instrumentation, and their media being not ready to use. All of the most commonly used rapid mycobacterial culture systems like BACTEC 460, BACTEC MGIT (Becton Dickinson Diagnostic Instrument Systems, Towson, MD, USA) (Huang et al., 2001; Pfyffer et al., 1997; Saitoh and Yamane, 2000; Sorlozano et al., 2009; Telenti et al., 1993; Tortoli et al., 1999; Turkkani et al., 2010), MB/BacT ALERT 3D (bioMérieux, Marcy l'Etoile, France) (Manterola

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et al., 1998; Saitoh and Yamane, 2000; Sorlozano et al., 2009), and ESP Culture System II (ESP II; Trek Diagnostics, Westlake, OH, USA) (Turkkani et al., 2010) use Middlebrook broth that requires the addition of oleic acid, albumin, dextrose, catalase (OADC), and selective antimicrobials before inoculation of the processed sample. These manipulations require extra time and effort, and increase the risk of contamination.

We have developed a ready-to-use, biphasic, rapid mycobacterial culture medium called TK Medium (SALUBRIS, Woburn, MA, USA) that enables early detection of mycobacterial growth by changing its color (Kocagöz, 2010). TK Medium includes egg and additional nutrients like glutamic acid and iron. Since the production process does not require heating the heat-susceptible vital factors contained in the egg, these probably make the medium richer than classical egg-based media, enabling better mycobacterial growth. The medium contains pH indicators, and the color change basically depends on pH change due to bacterial growth. Mycobacterial growth causes its original red color to turn yellow (Kocagöz et al., 2007; Pai et al., 2006; World Health Organization (WHO), Foundation for Innovative New Diagnostics (FIND), 2006). The color change occurs even before the colonies have grown sufficiently to become visible on the solid part of the medium. TK Medium has the advantage of differentiating mycobacterial growth from the growth of most common contaminants like fungi and Gram-negative bacilli because the growth of those contaminants causes the medium to change to green instead of yellow (Fig. 1). Although some Gram-positive bacteria may change the color of TK Medium to yellow, they rarely survive decontamination, and even if they do, they are inhibited by antimicrobials included in TK SLC, the selective form of the medium. TK Medium is designed as a biphasic medium to enable the visualization and isolation of individual colonies on the solid part and to visualize features like cord factor in the liquid phase (Fig. 2).

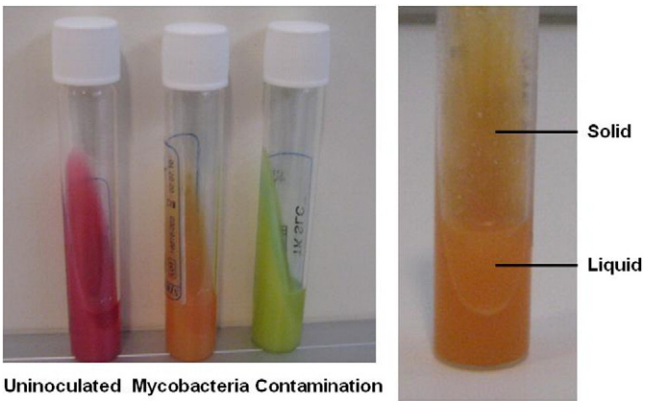

Fig. 1. Color changes that occur in TK Media by growth of mycobacteria and other (contaminant) organisms. Colonies on the solid part and cords of mycobacteria (in strains producing a cord factor) in the liquid part of the medium can be observed.

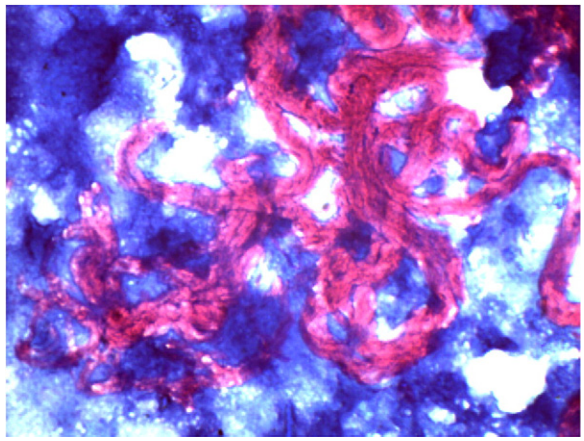

Fig. 2. Smear prepared from the liquid part of TK SLC and stained by Kinyoun. Long cords of AFB are observed indicating that the strain produces a cord factor.

Pure cultures of mycobacteria can be obtained by subculturing individual mycobacterial colonies if mixed organisms are obtained in the original culture. To lower the rate of contamination, TK Medium was supplemented with selective antimicrobials, polymyxin B, piperacillin, amphotericin B, nalidixic acid, and trimethoprim, to produce TK SLC. To eliminate the possibility that these antimicrobials may inhibit the growth of mycobacteria belonging to different species, we have previously inoculated 98 different species of mycobacteria (culture collection strains obtained from the American Type and Culture Collection [Manassas, VA, USA] or from DSMZ [Germany]) into TK SLC, and all of them have grown well without a sign of inhibition (unpublished data). The TK Anti Tb and PNB kit is used for simultaneous susceptibility testing and for *Mycobacterium tuberculosis* complex and MOTT (mycobacterium other than tuberculosis) differentiation. It contains a suspension tube, a dilution tube, a TK Medium growth control tube, and TK Media tubes containing isoniazid (INH) (0.2 μg/mL), rifampin (1.0 μg/mL), streptomycin (2.0 μg/mL), ethambutol (7.5 μg/mL), and *para*-nitro benzoic acid (PNB) (250 μg/mL). TK SLC and the TK Anti Tb and PNB Kit are stored at 4 °C, and their shelf-life is 6 months.

Since the growth in TK Media is detected by color change, it can be easily followed by visual evaluation and can be used in laboratories that have a regular 37 °C incubator. On the other hand, it has a very elaborate automated incubator reader, called Mycolor TK (SALUBRIS-technica) (Fig. 3). Mycolor TK detects and monitors the color changes (from red to yellow or to green). Mycolor TK provides growth curves, and its expert system predicts the type of growing microorganism.

This study was done to investigate the performance and advantages of the TK Rapid Mycobacterial Culture System (TK System) in daily use at the Yedikule Chest Diseases and Chest Surgery Education and Research Hospital, located in Istanbul, which is one of the largest hospitals taking care of

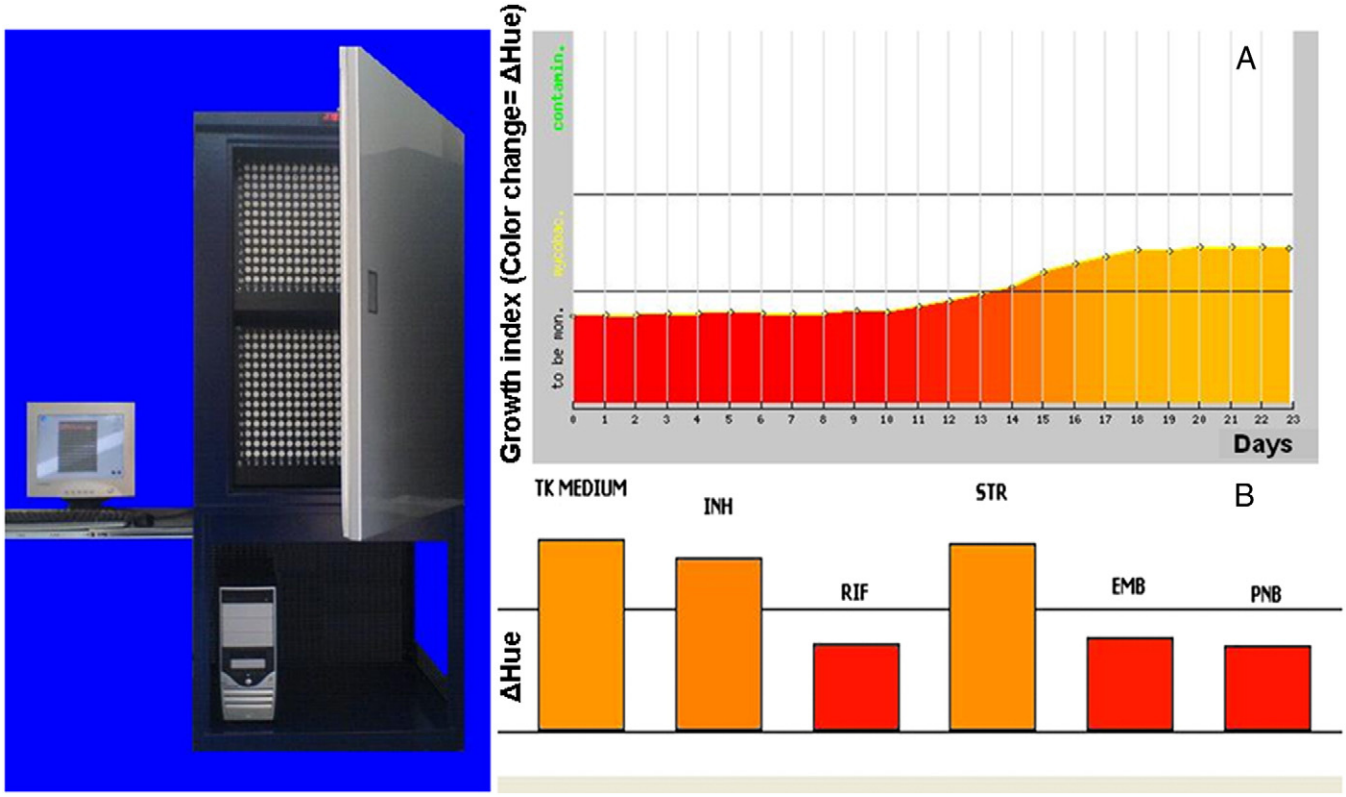

Fig. 3. Mycolor TK and the diagrams obtained in the instrument. A) Typical growth curve of a *M. tuberculosis* strain. B) The bar graph of the antibiotic susceptibility test of a *M. tuberculosis* strain resistant to INH and streptomycin.

tuberculosis patients in Turkey. This is the first large evaluation of the TK System after being introduced to clinical practice.

2. Materials and methods

A total of 16,303 clinical samples from patients suspected to have tuberculosis, submitted for microscopic examination and culture, during a 1-year period between May 2009 and May 2010, to the tuberculosis laboratory of Yedikule Chest Diseases and Chest Surgery Education and Research Hospital, Istanbul, Turkey, were evaluated. Sputum samples (91.5%) constituted most of the specimens followed by bronchoalveolar lavage (BAL) (7.3%) and other samples (1.2%) including tracheal aspirate, pleural fluid, pus, and biopsy samples.

Samples were decontaminated by the NaOH-NALC decontamination concentration (Kubica) method using a ready-to-use kit, Mycoprosafe (SALUBRIS). Decontamination solution contained 4% NaOH. Duration of decontamination was 15 min. From the processed samples, 500 μL was inoculated into LJ and TK SLC media; smears were prepared, stained by Kinyoun stain, and examined under a microscope for the presence of acid fast bacilli (AFB). Inoculated LJ media were incubated for 6 weeks at 37 °C, and growth were evaluated every day except the weekends. Growth detection time was recorded when colonies were visible on the slants. TK SLC tubes were monitored by Mycolor TK. Growth detection time for positive cultures, corresponded to the results of the instrument. All positive cultures were confirmed for the presence of AFB, by preparing a smear from the colonies on LJ and from the liquid portion of TK SLC and by microscopy examination.

For susceptibility testing and *M. tuberculosis* complex MOTT differentiation, 200 μL from the liquid portion of TK SLC was transferred to the suspension tube of the TK Anti Tb and PNB kit. After shaking with glass beads, 500 μL of the suspension was transferred to a dilution tube that contained 4.5 mL of liquid to prepare a 1:10 dilution. From this diluted suspension, 200 μL was inoculated into each tube. The tubes were placed into Mycolor TK which analyzes the growth in the control and drug-containing tubes. Mycolor TK reports the results as “susceptible” or “resistant.” Inhibition of growth in the PNB tube indicates that the isolate belongs to the *M. tuberculosis* complex group, and growth in PNB indicates MOTT. When more than 1 mycobacterial isolate was obtained from the same patient, only one of them was subjected to susceptibility testing and typing. Quality control for susceptibility testing was done by 2 studies. Thirty-six collection strains provided by the World Health Organization (WHO) with previously

known susceptibility patterns, which included 13 borderline rifampin-resistant strains with known *rpoB* mutations, were tested by the TK Anti Tb and PNB kit, and the susceptibility patterns were compared to the known results. Additionally, the hospital laboratory participated in the external quality control program organized by Refik Saydam, the National Public Health Agency, Tuberculosis Reference and Research Laboratory, Ankara, Turkey. In this program, the susceptibility to 4 major antituberculosis drugs of 20 *M. tuberculosis* strains was determined. Quality control, for the efficiency of TK PNB in differentiation of *M. tuberculosis* complex and MOTT, was done by determining the species of all MOTT and 55 randomly selected *M. tuberculosis* isolates by polymerase chain reaction amplification of the hsp65 gene and by restriction enzyme analysis (PRA), as described originally by Telenti et al. (1993).

Statistical analysis of mycobacterial isolation and contamination rates was done using McNemar test and comparative time-to-growth detection by *t* test.

3. Results

The results of microscopy and culture in LJ and TK SLC are summarized in Table 1. Among 16,303 samples included in the study, 1374 (8.42%) were AFB positive by microscopic examination. There was no growth in any type of media from 91 (0.56%) of the samples which were AFB positive by microscopy. In 2150 (13.04%) samples, mycobacteria were isolated in at least 1 type of medium. These belonged to 1571 patients (since for some patients more than 1 sample was positive in culture). While LJ isolated AFB from 1920 (11.69%) of all samples, TK SLC isolated 2070 (12.63%). The sensitivity of LJ and TK SLC was 89.3% and 96.3%, respectively (87.9% to 90.5% for LJ and 95.4% to 97.0% for TK SLC with a *P* value of 0.05). The difference was statistically significant as evaluated by McNemar test ($P < 0.0001$). No false-positive result was observed in either TK SLC or LJ, making the specificity of both media 100%. The positive and negative predictive values were 100% and 98.4% for LJ and 100% and 99.4% for TK SLC, respectively. It was possible to isolate *M. tuberculosis* in TK SLC from different types of samples including sputum, BAL, tracheal aspirate, pleural fluid, pus, and biopsy specimens. When growth was detected in TK SLC tubes by the Mycolor TK instrument, a smear was prepared from the liquid portion of the medium and was stained by Kinyoun's stain. In all of these smears, AFB was observed and no reincubation was required in any tube. Contamination of cultures by other organisms was observed in 878 (5.33%) LJ tubes and 90 (0.55%) TK SLC tubes. The difference was again statistically significant as evaluated by McNemar test ($P < 0.0001$). In all TK SLC tubes that turned green and whose contamination was indicated by Mycolor TK, contaminant organisms were detected by Kinyoun's staining. No mixed culture of mycobacteria and other organisms was observed in this study. Among 2150 mycobacterial isolates, only 17 (0.79%) were identified as MOTT and all other 2133 isolates belonged to the *M. tuberculosis* complex group. Time-to-growth detection varied between 3 and 28 days (average 15.57 days) in TK SLC and between 10 and 41 days (average 25.14 days) in LJ. The modes of time-to-growth detection (day on which the growth was most frequently detected) were 12 days for TK SLC and 25 days for LJ. The distribution of duration of time-to-growth detection for TK SLC and LJ is shown in Fig. 4. The difference between average growth detection times with TK SLC and LJ was 9.58 days (SD, ±4.04 days), which was statistically significant ($P < 0.001$) as evaluated by *t* test.

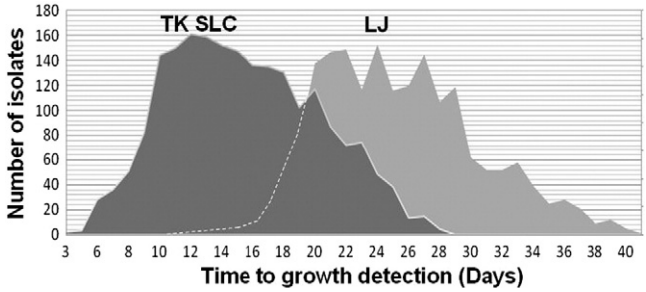

Fig. 4. Distribution of the number of isolates according to the duration of time-to-growth detection. Most of the isolates grew between 10 and 15 days in TK SLC with a mode of 12 days and between 21 and 29 days in LJ with a mode of 25 days.

A total of 1571 isolates, each one isolated from different patients, were subjected to antimycobacterial susceptibility testing using the Anti Tb and PNB Kit (Fig. 5); 1193 (75.93%) were susceptible to all 4 major antituberculosis drugs tested. INH resistance was detected in 293 (18.93%), rifampin resistance in 209 (13.30%), streptomycin resistance in 133 (8.47%), and ethambutol resistance in 83 (5.28%) isolates. The number of multidrug-resistant (MDR) isolates

Table 1
Comparison of microscopy, LJ, and TK SLC in detecting mycobacteria in clinical samples

	Number of positives among 16,303 samples	Rate of positivity among all (2150) positive cultures	Contamination (among 16,303 samples)	Average time-to-growth detection (days)	Mode of time-to-growth detection (days)
Microscopy	1374 (8.40%)	59.67%	NA[a]	NA	NA
LJ	1920 (11.69%)	89.30%	878 (5.33%)	25.14	25
TK SLC	2070 (12.63%)	96.27%	90 (0.55%)	15.57	12

[a] NA = Not applicable.

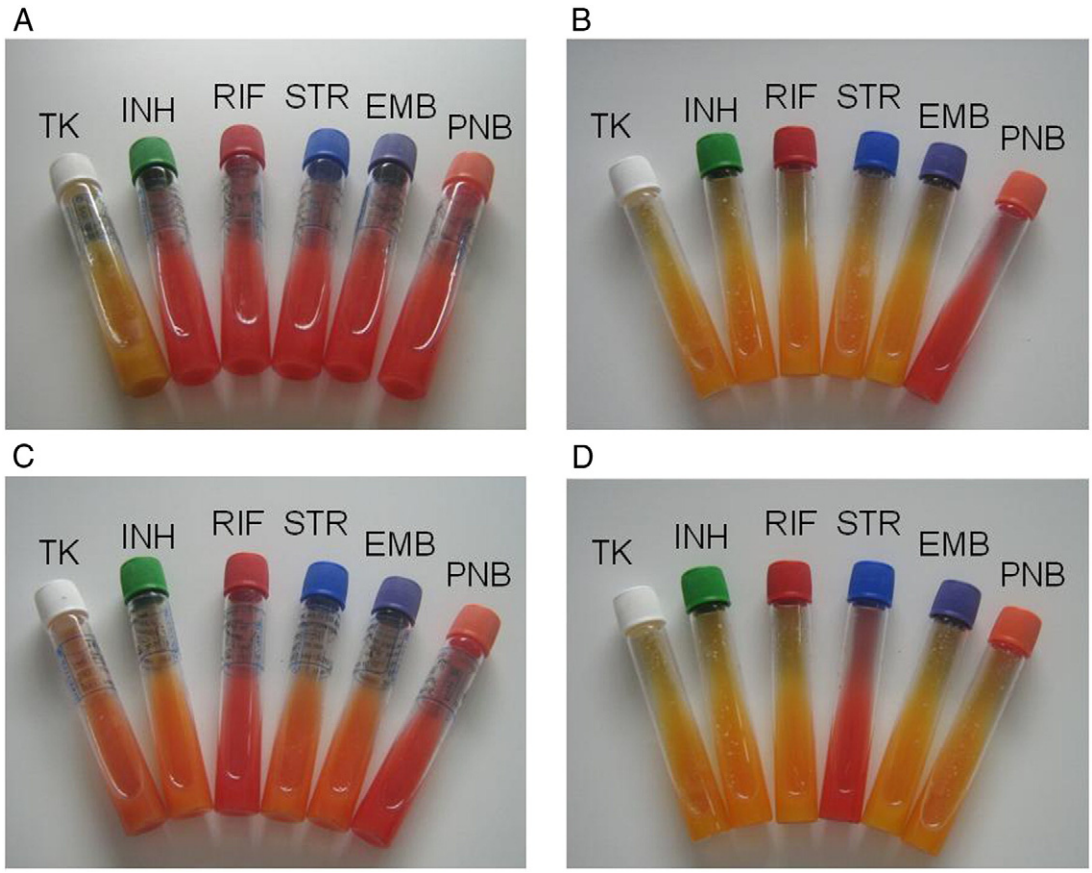

Fig. 5. Examples of susceptibility testing and typing results. Yellow indicates growth and red inhibition. A) *M. tuberculosis* susceptible to all drugs; B) *M. tuberculosis* resistant to all drugs; C) *M. tuberculosis* susceptible to rifampin, resistant to INH, streptomycin, and ethambutol; D) MOTT susceptible to streptomycin, resistant to INH, rifampin, and ethambutol (growth in PNB indicates that it is a MOTT species).

was 160 (10.18%); 58 of these MDR isolates were resistant to all 4 major drugs tested. Susceptibility testing and mycobacterium tuberculosis complex and MOTT differentiation results were obtained in 10.35 days, on average.

All susceptibility results, of the 36 *M. tuberculosis* WHO collection strains, were consistent with the susceptibility patterns reported by WHO except for 3 types of strains with borderline resistance to rifampin. In mutants containing *rpoB* 441Tyr and 497Phe mutations, rifampin resistance was not detected. Rifampin resistance was detected in 1 of 3 strains with *rpoB* 436Pro mutation. It was possible to detect resistance in borderline rifampin-resistant strains with *rpoB* 451Leu, 451 Ser, 456Leu, and 458Pro mutations.

The laboratory was also validated for susceptibility testing by an external quality control program. All susceptibility patterns of 20 *M. tuberculosis* strains tested were identified correctly for INH, rifampin, streptomycin, and ethambutol; only 2 strains with borderline resistance to ethambutol were reported as susceptible.

The species of 55 randomly selected strains among 2133 isolates identified as *M. tuberculosis* complex by their inhibition in TK PNB and all 17 isolates identified as MOTT were determined by PRA of *hsp65*. Among 17 MOTT strains, 7 were identified as *M. abscessus*, 5 as *M. fortuitum*, and 1 each of *M. kansasii*, *M. chelonae*, *M. gordonae*, *M. murale,* and *M. xenopi*. The species, of all 55 strains identified as *M. tuberculosis* complex by TK PNB, were also confirmed by PRA. The 36 WHO collection strains and 20 external quality control strains known as *M. tuberculosis* were also correctly identified by TK PNB, making the total number of correctly identified *M. tuberculosis* strains equal to 111.

4. Discussion

Culture continues to be the gold standard method for the diagnosis of tuberculosis. Additional to its ability to establish a definite diagnosis, culturing mycobacteria also enables the determination of antituberculosis drug susceptibility and allows to perform epidemiologic studies (de Waard and Robledo, 2007; Pai et al., 2006; World Health Organization (WHO), Foundation for Innovative New Diagnostics (FIND), 2006). Since classical media like LJ require 3 to 6 weeks for the detection of growing

M. tuberculosis colonies, rapid culture systems that can detect mycobacterial growth early were developed. All of these systems, including BACTEC 460, BACTEC MGIT, MB/BacT ALERT 3 D, and ESP Culture System II, use Middlebrook broth as their culture medium. The only difference between these systems is the technique for detection of microbial growth. Since the shelf-life of Middlebrook broth is limited, these culture systems provide incomplete Middlebrook medium in tubes or flacons and OADC supplements and selective antimicrobials lyophilized in flacons. Prior to use, it is necessary to rehydrate the supplements and add them to the culture tubes. In order to prepare the media for antimicrobial susceptibility testing, lyophilized antituberculosis drugs should also be rehydrated and added to the Middlebrook media. These manipulations require extra time and effort, and increase the risk of contamination. It may also be a problem for standardization of antimicrobial susceptibility testing since the concentration of antituberculosis drugs depends on the correct preparation and addition of these drugs by the user. All these culture systems, except BACTEC MGIT, depend on automated instrumentation; microbial growth cannot be monitored by visual evaluation. MGIT tubes can alternatively be monitored by visual inspection using a Wood's lamp.

All TK Media are ready to use. They include everything necessary for their purpose. TK SLC medium which is used for inoculation of clinical samples contains selective antimicrobials to minimize contamination. The TK Anti Tb and PNB kit contains media with antituberculosis drugs for susceptibility testing and PNB for *M. tuberculosis* complex and MOTT differentiation. Since they are all ready to use, they do not require preparation work prior to use, which eliminates the risk of contamination due to these manipulations and enable the standardization of antituberculosis drug susceptibility tests by preadjusting the concentration of drugs.

Culturing in both LJ and TK SLC was more sensitive than microscopic examination in the diagnosis of tuberculosis, as expected (Table 1). TK SLC was also superior to LJ by isolating mycobacteria in 150 (6.97%) more specimens among a total of 2150 isolates. Duration of time-to-growth detection was also significantly lower in TK SLC, being 15.57 days compared to 25.14 days in LJ on average. Growth in most of the samples was detected between the 10th and 15th day with a mode of 12 days as opposed to 25 days in LJ (Fig. 3). In the laboratory where the study was done, everyday 70 to 100 samples are processed and inoculated into culture media. Specimen inoculated on LJ media are kept incubated for 6 weeks. There are about 3000 LJ tubes that should be followed all the time, which creates an immense workload. LJ tubes were evaluated everyday during this study to determine the exact growth detection time; however, in routine practice, the tubes are evaluated once or twice a week, which delays the growth detection time. The observed growth detection time for LJ in this study is probably shorter than the real growth detection time in routine daily application which should be considered as a trial bias.

Since TK SLC medium can be evaluated visually, it may be well suited for laboratories where automated instruments are unaffordable. Due to the high workload of this study, it was not possible to evaluate all TK SLC tubes everyday visually and the average growth detection time according to visual evaluation was not obtained. However, when a limited number of TK SLC tubes were visually evaluated, it was usually possible to detect growth on the same day as the growth indicated by the Mycolor TK instrument. In future studies, a larger number of inoculated TK SLC tubes should be evaluated visually, preferably by more than 1 person, in a double-blind manner, in order to determine the average growth detection time by visual evaluation.

The duration of time-to-growth detection of the TK system was comparable to that of other rapid mycobacterial culture systems. Huang et al. (2001) compared the BACTEC 460 radiometric system and BACTEC MGIT and determined the average duration of time-to-growth detection at 16.6 and 13 days, respectively. In the study done by Pfyffer et al. (1997) comparing BACTEC MGIT, BACTEC 460, and LJ, time-to-growth detection was 9.9, 9.7, and 20.2 days for *M. tuberculosis* complex and 11.9, 13.0, and 22.2 days for MOTT, respectively. In a multicenter study done by Tortoli et al. (1999), average time-to-growth detection for BACTEC MGIT, BACTEC 460, and LJ was 13.3, 14.8, and 25.6 days, respectively. Williams-Bouyer et al. (2000) compared ESP Culture System II with BACTEC MGIT and determined a time-to-growth detection of 15.8 and 12.5 days, respectively. Manterola et al. (1998) determined an average time-to-growth detection of 14.2 days for the MB/BACT system, 11.7 days for BACTEC 460, and 26.1 days for egg-based solid media. Sorlozano et al. (2009) compared BACTEC MGIT, MB/BacT ALERT 3D, and LJ, and determined a duration of time-to-growth detection of 15.1, 20.2, and 32.4 days, respectively. In a study done by Saitoh and Yamane (2000), BACTEC MGIT detected mycobacteria in 20 days and MB/BacT in 17 days on average.

One major drawback of rapid culture systems that use Middlebrook broth is their contamination rate. Williams-Bouyer et al. (2000) determined the contamination rate for BACTEC MGIT as 17.1% and 18.9% for ESP Culture System II. Huang et al. reported 13.2%, and Saitoh and Yamane reported a 20.1% contamination rate for BACTEC MGIT. Tortoli et al. determined a 10.0% contamination rate for BACTEC MGIT in a multicenter study (Huang et al., 2001). The high contamination rate may be due to the expertise of the laboratory on media preparation since Middlebrook media requires the addition of OADC supplement and selective antimicrobials prior to its use, and these manipulations may increase contamination. This may be observed in the study of Pfyffer et al. performed at 3 centers. Although the centers applied the same protocols,

the authors found contamination rates of 2.0%, 13.8%, and 6.1% for BACTEC MGIT (Pfyffer et al., 1997). In this study, TK SLC had a contamination rate of just 0.55%. We believe that this is not due to over-decontamination of the specimens since the contamination rate for LJ was 5.33% for the same specimens. First of all, TK SLC is a ready-to-use medium and does not have a risk of contamination due to the addition of supplements. Secondly, TK SLC includes piperacillin instead of azlocillin which is present in the antimicrobials of the other rapid culture systems. *Pseudomonas* is a frequent contaminant of mycobacterial culture media, and piperacillin has a better antipseudomonal activity than azlocillin. This may also reduce the contamination rate compared to other rapid culture media. Further studies comparing the TK Culture System with other rapid mycobacterial systems, for direct comparison of mycobacterial isolation and contamination rates in these systems, are required.

In this study, TK PNB identified correctly all 17 MOTT isolated from patients' samples and 111 *M. tuberculosis* complex strains (55 clinical isolates and 56 collection strains). Although according to the results of this study TK PNB seems pretty reliable in differentiating *M. tuberculosis* complex and MOTT strains, further evaluation involving MOTT species other than the few MOTT species isolated in this study will be beneficial.

The TK Culture System proved also to be reliable in susceptibility testing. It passed an external quality control test with an accuracy of 98%. It also correctly identified the susceptibility of all 36 WHO collection strains except for the rifampin resistance of a few borderline-resistant rifampin strains. Since the MIC of these strains is lower than 1 μg/mL for rifampin, the clinical relevance of their resistance can be debated. Further comparative susceptibility studies with standard methods, involving larger number of strains, will be beneficial in the validation of breakpoint concentrations of antituberculosis drugs used in TK susceptibility culture media.

In 2008, the Tuberculosis General Directorate of the Ministry of Health reported a resistance rate in Turkey for INH, rifampin, streptomycin, and ethambutol of 13.6, 6.6, 7.5, and 4.3, respectively. MDR strains constituted 5.3% of all isolates (Turkkani et al., 2010). In this study, the resistance rate of INH (18.93%) and that of rifampin (13.30%) were higher along with that of MDR (10.18%) compared to the general resistance rate in Turkey. This is an expected result since the hospital, where the study was done, hospitalizes complicated patients with previous history of treatment failure.

With its ready-to-use media and automated reader, the TK Culture System may be a practical system which shortens the time required for both culture and susceptibility results. It may save a lot of time and effort in tuberculosis laboratories with high workloads. TK Media can also be used in laboratories with low numbers of samples since they can also be evaluated visually. According to the results of this study, it is a reliable system with higher rates of isolation of mycobacteria compared to LJ and with very low rates of contamination. Finally, the TK Culture System is expected to lower the costs of automated mycobacterial culture systems (when the hospital, where this study was performed, switched from BACTEC MGIT to the TK Culture System, it reduced its cost of rapid mycobacterial culture by 60%). Having all these features, the TK Culture System may be used in clinical laboratories with limited resources and also in laboratories with elaborate capabilities. Future studies in different settings may be beneficial for further evaluation of the efficiency of the TK Culture System.

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