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Full Length Research Paper

Comparison of the VersaTrek and BACTEC MGIT 960 systems for the contamination rate, time of detection and recovery of mycobacteria from clinical specimens

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The most commonly used conventional Lowenstein Jensen (L.J) culture method requires at least 6 to 10 weeks of incubation due to the slow growth rate of the *Mycobacterium tuberculosis* complex. Today, there are liquid automated systems to detect mycobacteria than faster conventional LJ culture method. In this study, we aimed to compare of the VersaTrek system and BACTEC MGIT 960 system for the contamination rate and recovery of mycobacteria from clinical specimens. The recovery rates of mycobacteria were detected as 4% (2/50) for both of the VersaTrek and MGIT 960 systems. No statistically significant difference was detected for the recovery rates of mycobacteria in both of the systems (p>0.05). Two strains were detected on 12 and 15 day in MGIT 960 system. Two strains were detected on 15 and 20 day in VersaTrek system. No statistically significant difference was detected for the time of detection of mycobacteria in both of the systems (p>0.05). The contamination rates were found as 10% (5/50) and 6% (3/50) in MGIT 960 and VersaTrek, systems respectively (p>0.05). No statistically significant difference was detected for the contamination rates for both of the systems. Antituberculosis resistance tests were performed using with antituberculosis agents in MGIT 960 and VersaTrek systems. The two strains were found sensitive against to streptomycin, isoniazid, rifampicin and ethambutol in MGIT 960 system. The two strains were found sensitive against to isoniazid, rifampicin and ethambutol in VersaTrek system. Streptomycin was not used in VersaTrek system because there is no standardization for streptomycin. We also examined the time of detection of 5 confirmed *M. tuberculosis* complex members in MGIT 960 and VersaTrek systems. When the time of detection in MGIT 960 and VersaTrek systems were compared, no statistically significant difference was found (p>0.05). In conclusion, no substantial difference has been observed between MGIT 960 and VersaTrek systems for *M. tuberculosis* isolation and contamination rate. We believe that VersaTrek media, which is going to be put into use in Turkey, is easy for application and follow up, gives rapid and sensitive results and that it can be used in routine alike MGIT media.

Key words: VersaTrek, MGIT 960, *M.tuberculosis.*

INTRODUCTION

Tuberculosis is a major health problem in the world. Bacteriological confirmation plays a key role in the diagnosis of tuberculosis (TB). The most commonly used conventional Lowenstein Jensen (L.J) culture method requires at least 6 to 10 weeks of incubation due to the slow growth rate of the *Mycobacterium tuberculosis* complex (Salfinger and Pfyffer, 1994). There is no need to wait for the culture results to start the treatment in new cases where acid-resistant bacilli (acid resistant bacilli: ARB) is found positive in sputum examination. However, the same situation does not apply to ARB negative cases. Although radiological and clinical findings provide benefit for treatment, what is essential is carrying out bacteriological examinations (Atas et al., 2003).

Today, Lowenstein-Jensen (L.J) medium is considered

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as the golden standard for the microbiological diagnosis but it requires a long time, like 4 to 6 weeks. Therefore, BACTEC system (BD Biosciences, Sparks, MD) which is faster than conventional L.J medium is also used as golden standard with L.J medium (Pfyffer et al., 1997; Palaci et al., 1996). Among the methods utilizing liquid media, the half-automated radiometric BACTEC 460 TB system (BACTEC 460) is widely accepted as the reference standard. This system, however, is also known for some well-established limitations, which include problems with the use of radioactive material, cumbersome manual loading and unloading, potential hazard of needle stick injury, risk of cross contamination, and lack of computerized data management. Recently, the BACTEC Mycobacteria Growth Indicator Tube System (BACTEC 960/MGIT), a newly developed non radiometric, fully automated, continuously monitoring system, was introduced as an alternative to the radiometric BACTEC460 for growth and detection of mycobacteria (Williams-Bouyer et al., 2000; Augustinowic-Kopec et al., 2002). There is silicon that contains oxygen- sensitive ruthenium metal complex in Middlebrook 7H9 liquid medium and its bottom sections. When mycobacterium or other micro-organisms grow, fluorescence occurs against UV rays as a result of the use of oxygen and the amount of fluorescence is evaluated as the growth index (Somoskovi et al., 2000).

The technology of the VersaTrek/ESP culture system is based on the detection of headspace pressure changes within a sealed bottle. It monitors changes in either gas production or gas consumption due to microbial growth (every 24 min). A special algorithm has been developed for detection of very slow growing mycobacteria. This system automatically incubates and continuously monitors culture bottles inoculated with specimens possibly containing mycobacteria. The bottles are incubated during 42 days (Gravet et al., 2010).

In this study, we aimed to compare of the VersaTrek system and BACTEC MGIT 960 system for the contamination rate and recovery of mycobacteria from clinical specimens.

MATERIALS AND METHODS

Fifty clinical samples (sputum, gastric and bronchoalveolar lavage fluids, urine, CSF (Cerebrospinal fluid), abscesses, pleura, peritoneal and ascites fluids) which have been sent to Microbiology and Clinical Microbiology laboratory of Cerrahpasa Faculty of Medicine were included in this study. Clinical specimens received for mycobacterial culture were processed by standard laboratory protocols. A quantity of 4% NaOH solution is added to 50 ml sterile screw cap tubes (Falcon tubes) containing the clinic samples for 15 to 20 min. At the end of the waiting period, phosphate buffer solution was added to the falcon tubes. The tubes containing the phosphate buffer were centrifuged at 3000 x g for 15 to 20 min. Then the upper liquid section was discharged and 1 to 2 ml of sterile phosphate buffer was added to the sediment. The sediments were inoculated on to L. J slants, MGIT 960 tubes containing growth supplement and PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) and VersaTrek Myco bottles containing growth supplement and PVNA (polymyxin B, vancomycin, nalidixic acid, amphotoricin B) antibiotic mixture (Trek Diagnostic Systems, Westlake, OH, USA). All steps were performed in accordance with the manufacturer's recommendations (Trek Diagnostic Systems, 2000). Bottles were monitored continuously for growth and an aliquot was removed for identification when a positive culture was detected. In both methods, a series of algorithms were used to determine presumptive positivity and to alert the operator to the presence and location of positive tubes. Any sample identified as positive by the instrument (VersaTrek or BACTEC MGIT 960 System) was removed from it, and to be confirmed, a smear from liquid media was prepared and tested for the presence of AFB by Ziehl- Neelsen (ZN) staining. If staining confirmed the presence of AFB, the result was considered positive (true positive by the instrument). If staining did not reveal AFB, a subculture on L. J was performed to control growth and purity, and the tube was re-incubated at 37°C in a heater (not in the instrument) for a further 42 days, after which the ZN staining was repeated. Samples showing mycobacterial growth in L.J and/or presence of AFB by ZN staining after the 42 day incubation were considered positive (true positive by the instrument). Any sample initially identified as positive by the instrument but showing no presence of AFB by staining or mycobacterial growth in L.J, was considered negative (false positive by the instrument). Samples were considered negative if mycobacterial growth was not detected by any automated method or no growth was observed in L.J medium after 45 days (true negative in all methods). Identification using PNBA: It has been reported that the growth of MTB isolates is inhibited by PNB 500 mg/ml whereas NTM are resistant to this concentration. The PNB stock solution was prepared to ensure a final concentration of 500 mg/ml in the MGIT vial. This stock solution was aliquoted and stored at -20.0. The PNBA test was performed by inoculating the positive culture into two MGIT tubes with and without PNBA and incubated in the MGIT 960 system (Giampaglia et al., 2007).

Resistance test was performed on the samples detected as positive in MGIT 960 and VersaTrek Myco bottles. For MGIT, streptomycin, isoniazid, rifampicine and ethambutol were tested in BACTEC MGIT 960 tubes containing antibiotics, isoniazid, rifampicine and ethambutol were tested in VersaTrek Myco bottles. We also examined the time of detection of 5 confirmed *M. tuberculosis* complex members in L.J medium, MGIT 960 and VersaTrek systems. We used the cultured 5 *M. tuberculosis* complex members on the L.J slants. We scraped with a sterile loop as many colonies as possible from growth no more than 14 days old, trying not to remove any solid medium. We suspended the colonies Middlebrook 7H9 Broth. We vortexed the suspension for 2 to 3 min to break up the larger clumps. The suspensions were adjusted to A 1.0 McFarland standart in turbidity. We let the suspension sit for 20 min without disturbing. We adjusted the suspension to a 0.5 McFarland turbidity standart. We followed the manufacturer's recommendations for other steps to inoculate MGIT 960 and VersaTrek systems. We also inoculate to the L.J slants.

Statistics

Statistical analyses were performed with the UNISTAT 5.0 statistical package for Windows (UNISTAT Ltd., London, UK) and SPSS 10.0 for Windows TM (SPSS Inc., Chicago, IL).

RESULTS

The recovery rates of mycobacteria were detected as 4%

Table 1. Antituberculosis resistance test result with growth strains in MGIT 960 and VersaTrek systems.

Patient number	Streptomycin	Isoniazid	Rifampicin	Ethambutol
1. Patient (MGIT)	Susceptible	Susceptible	Susceptible	Susceptible
2. Patient (MGIT)	Susceptible	Susceptible	Susceptible	Susceptible
1. Patient (VersaTrek)	Not Performed	Susceptible	Susceptible	Susceptible
2. Patient (VersaTrek)	Not Performed	Susceptible	Susceptible	Susceptible

Table 2. Time to detection of strains cultured from L.J.

Patient number	MGIT 960 (days)	VersaTrek (days)
1. Patient	5	6
2. Patient	6	6
3. Patient	5	7
4. Patient	5	6
5. Patient	7	6

(2/50) for both of the VersaTrek and MGIT 960 systems. The same 2 patients were positive in both of the systems. No statistically significant difference was detected for the recovery rates of mycobacteria in both of the systems ($p>0.05$). The time of detection for two strains in MGIT 960 and VersaTrek systems were evaluated. The two strains were detected on 12 and 15 day in MGIT 960 system. The two strains were detected on 15 and 20 day in VersaTrek system. No statistically significant difference was detected for the time of detection of mycobacteria in both of the systems ($p>0.05$).

The contamination rates were found as 10% (5/50) and 6% (3/50) in MGIT 960 and VersaTrek, systems respectively ($p>0.05$). No statistically significant difference was detected for the contamination rates for both of the systems. Antituberculosis resistance tests were performed using with antituberculosis agents in MGIT 960 and VersaTrek systems. The two strains were found sensitive against to streptomycin, isoniazid, rifampicin and ethambutol in MGIT 960 system. The two strains were found sensitive against to isoniazid, rifampicin and ethambutol in VersaTrek system. Streptomycin was not used in VersaTrek system because there is no standardization for streptomycin (Table 1). We also examined the time of detection of 5 confirmed *M. tuberculosis* complex members in MGIT 960 and VersaTrek systems. When the time of detection in MGIT 960 and VersaTrek systems were compared, no statistically significant difference was found ($p>0.05$) (Table 2) .

DISCUSSION

It is certain that all studies that would increase *M. tuberculosis* isolation and shorten its period of detection in the culture could facilitate diagnosis and treatment. The most significant studies on this subject were realized by the introduction of liquid mediums to use. The novel and major developments in mycobacteriology laboratory technologies were achieved in recent years as a result of these.

There was no literature when we evaluated the studies which comparing MGIT 960 and VersaTrek systems. There were very few studies comparing VersaTrek system and L.J medium. Gravet et al. (2010) compared solid culture with VersaTrek for the time needed for detection of mycobacteria and found the time was significantly shorter for the VersaTrek with a good recovery rate. For isolates recovered in both systems, mean time of detection was found respectively 19.1 and 35.6 days for liquid and solid cultures. Mycobacteria identification may be determined using nucleic acid probs directly in Myco VersaTrek or in the solid medium. They concluded that VersaTrek system offers a faster diagnosis and is an alternative to other instruments using liquid culture.

Falconi et al. (2008) compared the recovery rate and mean time to detection (TTD) of mycobacteria of 2 culture media: the VersaTrek system and the L.J medium. The recovery rates were 84.8% (168/198) for the VersaTrek system and 89.4% (168/188) for L.J ($p=0.2$); while the contamination rates were 4.2% for the VersaTrek system and 7.4% for L.J ($p<0.001$). The TTDs for *M. tuberculosis* were 19.8 ± 11.2 day for the VersaTrek system and 27.3 ± 10.2 day for L.J ($p<0.001$). They concluded that the VersaTrek system significantly reduces the TTDs of mycobacteria detection, which is clinically relevant. In a study performed by Tortoli et al. (1999), the average isolation period of mycobacteria were found 13.3 days in MGIT. Primary isolation of *M. tuberculosis* from smear positive sputum samples by the VersaTrek system took 19.8 ± 11.2 days (Falconi et al., 2008), by the Biphasic system (Middlebrook 7H11 agar slant+Middlebrook 9H broth) it took 21 ± 4.4 days (Ghatole et al., 2005), by MGIT 960 it took 9 (7-11) days (Casal et al., 1997), 11.9 days (Diraa et al., 2003) and 12.6 days (Somoskövi et al., 2000), by the MB-Check culture system (liquid phase), it took 14.8 ± 8.0 days (Palacious et al., 1997) in various studies. Mean time to detect Mtb from smear positive pulmonary sample on L.J slants has been found to be in the range of 19-24 days by most workers (Diraa et al., 2003; Palacious et al., 1997).

The recovery rates of mycobacteria were detected as 4% (2/50) for both of the VersaTrek and MGIT 960 systems in our study. The same 2 patients were positive in both of the systems. The two strains were detected on 12 and 15 day in MGIT 960 system. The two strains were detected on 15 and 20 day in VersaTrek system. We also examined the time of detection of 5 confirmed *M. tuberculosis* complex members in MGIT 960 and VersaTrek systems. When the time of detection in MGIT 960 and VersaTrek systems were compared, no statistically significant difference was found ($p>0.05$). Our recovery rates were lower for both of the automated systems. Because of our hospital is not a specialized hospital for tuberculosis diagnosis and treatment. The doctors of hospital clinics suspect from every patient to have tuberculosis or not. Our recovery rate was 4.6% for 4500 average patients in our laboratory per year. For this reason low recovery rate was normal for our laboratory. The MGIT 960 system is a little bit faster than VersaTrek system for the recovery of mycobacteria but we did not detect a statistically significant difference between the two systems.

Ilgazli et al. (1988) found the contamination rate of MGIT medium as 11% in a study performed with 100 samples in zmit Tuberculosis Control Regional Laboratory in 1998. In a study performed by Oztürk et al. (2000) with 118 samples during the period October 1997 to June 1998 in GATA Haydarpasa Teaching Hospital, the contamination rate of MGIT medium was found 17.8%. They stated that a disadvantage of the liquid mediums were that their contamination rates were high. Pfyffer et al. (1997) found the contamination rates as 2, 13.8 and 6.1% respectively in a study carried out in three centres. Cornfield et al. (1997) lowered the contamination rate from a previously self-determined 29 to 12% by changing the sampling method. These researchers thought that these high contamination rates in MGIT method might have been caused by glycerol and dextrose used to enrich the medium.

The contamination rates were found as 10% (5/50) and 6% (3/50) in MGIT 960 and VersaTrek, systems respectively ($p>0.05$). Our contamination rate results for MGIT system is in accordance with above studies. The high contamination rates in MGIT system can be depend on the manual procedures and the enrichment of MGIT medium with glycerol and dextrose. Even there was no statistically significant difference between MGIT 960 and VersaTrek systems, we are aware of the advantage of using VersaTrek system because of safe manual processing of bottles.

We detected no difference for antituberculosis resistance tests using with antituberculosis agents in MGIT 960 and VersaTrek systems. The two strains were found sensitive against to streptomycin, isoniazid, rifampicin and ethambutol in MGIT 960 system. The two strains were found sensitive against to isoniazid, rifampicin and ethambutol in VersaTrek system. Streptomycin was not used in VersaTrek system because of there is no standardization for streptomycin. We detected no difference between two systems except streptomycin.

In conclusion, no substantial difference has been observed between MGIT and VersaTrek media for *M. tuberculosis* isolation and contamination rate. We believe that VersaTrek media, which is going to be put into use in Turkey, is easy for application and follow up, gives rapid and sensitive results and that it can be used in routine alike MGIT media.

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