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VersaTREK® for Use in Determination of Fungemia

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ABSTRACT

With the increasing number of patients with complex clinical conditions and undergoing complex medical therapy, more unusual infectious disease processes have emerged. Fungal pathogens as agents of a variety of infections, including fungemia, are part of this trend. Fungi are now found much more frequently as the cause of both community and hospital-acquired infections. Major predisposing factors leading to these infections include immunosuppression, use of broad-spectrum antibiotics, use of central venous catheters (especially in association with the administration of hyperalimentation solutions), and more aggressive attempts to prolong the survival of patients with complicated, serious disease. Recent data suggest that fungemias are more prevalent than anaerobic bacteremias. There are also frequent reports of illnesses associated with a long list of saprophytic and environmental molds and yeasts including dematiaceous fungi, multiple species of *Candida, Trichosporon beigelii, Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp.[1]

Detection of fungemia can be a labor intensive process with a high risk of culture contamination due to repeated specimen manipulation. Routinely, cultures are checked on a limited basis (daily or less in frequency) and an additional delay in detection is recognized as a result of blind subculturing from broth cultures. The ability to use a continuously monitoring blood culture analyzer could eliminate these problems and provide improved patient care by faster detection and ultimately isolate identification.

BACKGROUND

The VersaTREK® (VT) Microbial Detection System is FDA-cleared for use in the recovery of bacteria and yeast from blood and normally sterile body fluids. Additionally, the system has the flexibility to detect Mycobacteria and perform first-line drug testing for *Mtb* isolates. Although the system is not specifically cleared for fungi, our laboratory has recovered numerous isolates with the VT system over the years. The purpose of our study was two-fold, 1.) to determine which media best supports the recovery of fungi and yeast in the VT system (VT REDOX® 1 vs. VT Myco) and 2.) to evaluate the performance of the system with regard to time-to-detection (TTD) of both yeasts and fungi. The use of the analyzer for this function would reduce unnecessary hands on time (increase productivity), reduce time to detection (in most cases by the continuous monitoring by the analyzer) and reduce the risk of contamination (by reducing the amount of broth media sub-culturing) without reducing the sensitivity of the test.

METHODS

It is extremely difficult to evaluate a blood culture instrument using patient specimens as the vast majority yield negative results. Therefore, a procedure for evaluating the ability of the blood culture media to support growth AND the analyzer to detect the growth of pathogens using seeded specimens is necessary.

More than one hundred isolates, both patient isolates and ATCC reference strains, of yeasts and molds were used, along with actual patient specimens that were positive for yeast or molds for the study. Dilutions were made in sterile water to achieve an inoculum of <300 cfu/ml (as outlined in the quality control section of the VersaTREK Technical bulletin). In addition to the organism suspension, 5.0 ml of sterile human blood was inoculated to simulate clinical conditions in an aerobic blood culture bottle and 0.5 ml was inoculated into the MYCO bottles. The aerobic blood culture bottle was inoculated and placed on the analyzer in the same manner as routine blood cultures. The length of incubation (LOI) was extended to 28 days (the typical duration of a fungus culture). The MYCO bottle (used for AFB detection) inoculation was modified from the usual protocol by eliminating the use of PVNA (antimicrobial suspension designed to reduce/eliminate the growth of bacteria and fungi). The Growth Supplement (GS) however, was still used in the validation.

RESULTS

Table 1 lists the organisms tested in this study and the range of time-to-detection of the organisms.

During the initial phase of the testing, there was some trial and error in obtaining the correct dilution (McFarland Standard) in order to achieve the appropriate inoculum size as the current literature only defines dilutions for bacteria. Yeast/fungal spores are much larger than bacteria which affects the initial turbidity needed to realize the correct inoculum. Data was included for tests where the inoculum was less than 10 cfu/ml but growth was detected. If the inoculum size was greater than 300 cfu/ml, the test was repeated with the correct suspension. (For some molds the inoculum size was greater than 300 cfu/ml yet included in the study. These isolates were difficult to achieve the correct inoculum size but were included to demonstrate the ability of the analyzer to support and detect the growth of the particular organism.)

Four of the organisms tested failed detection by the VersaTREK system: *Cryptococcus albidus, Cryptococcus laurentii, Beauvaria sp.* and *Candida zeylanoides* (no growth was confirmed via subculture plate). This could be due to the fact that the current media formulation does not support the growth and/or that the temperature of the instrument was not optimal for growth of these organisms. For this reason, our protocol will now include a plated culture of the sediment from the specimen (blood collected in a 10 ml SPS vacutainer tube).

Table 1: Organisms Tested and their Time-to-Detection Range

Organism	Number of isolates tested	Range of TTD: Aerobic bottle (hours)	Range of TTD: MYCO bottle (hours)
Candida albicans	23	20.5 – 39.5	23.2 – 43.4
Candida glabrata	15	18.4 – 33.6	20.4 – 50.8
Candida kruseii	3	22.3 – 26.4	33.6 – 36
Candida kefyr	3	20 – 36	25.5 (2 not detected)
Candida parapsilosis	13	23.4 – 39.5	25.9 – 54.7
Candida tropicalis	4	17.8 – 21.8	23.1 – 25.6
Candida guilliermondii	2	27.4 – 36.8	33.1 – 37.2
Cryptococcus neoformans	4	17.8 – 21.8	67.2 – 71.9
Rhodotorula rubra	2	No growth	127.2
Aspergillus fumigatus	4	26.9 – 34.5	31.2 – 36
Blastomyces dermatitidis	2	No growth	264 – 384
Saccharomyces cerevisiae	3	19 – 62.6	25 – 28.8
Cryptococcus albidus	1	No growth	No growth
Cryptococcus laurentii	1	No growth	No growth
Paecilomyces spp.	1	102.4	60
Pseudozyma aphidis	1	29.8	Patient isolate
Penicillium spp.	8	22.2	25.6 – 429
Alternaria spp.	4	15.3 – 73.3	16.7 – 76.8
Aspergillus niger	1	27.6	31.5
Cladosporium spp.	1	No growth	62.8
Fusarium spp.	1	Patient isolate	144
Cunninghamella spp.	1	148.8	27.1
Syncephalastrum spp.	1	25.7	30.7
Sporothrix schenckii	1	99.7	96.7
Exophiala jeanselmei	1	201.6	314.4
Rhizopus spp.	1	189.6	285.6
Geotrichum spp.	1	50.4	51.4
Candida zeylanoides	1	No growth	No growth
Beauveria spp.	1	No growth	No growth
Scopulariopsis spp.	1	76	97.2
Trichosporon beigelii	1	13.1	16.3

(Diagnostic sensitivity: 96%)

DISCUSSIONS AND CONCLUSIONS

Due to the high mortality rate from fungemia, the expeditious detection and identification of fungi from a patient's blood can have great diagnostic and prognostic importance. Risk factors for fungemia and collecting fungal blood cultures include, but are not limited to, fever of unknown origin, extremes of age, immunosuppression, and those individuals with burns or indwelling intravascular devices.[2]

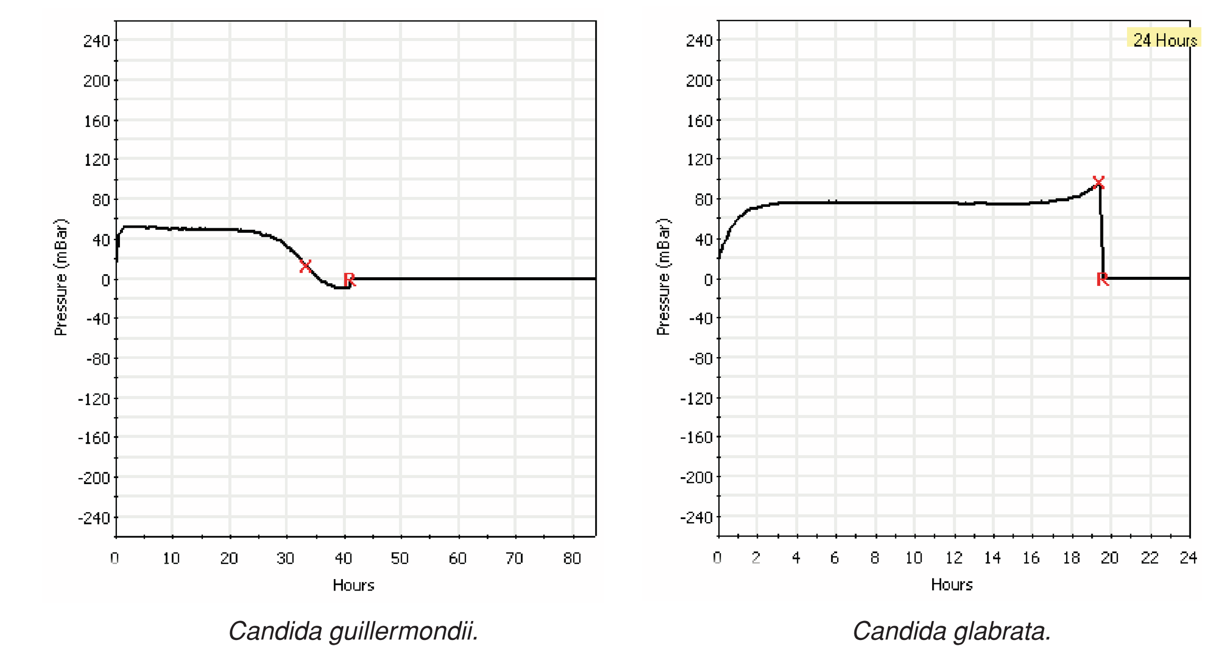
Though most fungemias are caused by yeast and most isolates were detected in the REDOX 1 (aerobic blood culture bottle) within the usual 5 day incubation period, there is the potential for the infectious agent to be a slower growing mold, of which some had better recovery and faster detection in the MYCO bottles. For this reason, we have decided on the following protocol to be used when a fungal blood culture is ordered by a clinician.

- Collect one 10 ml Vacutainer tube with SPS anticoagulant
- Transfer 5 ml of blood to a REDOX 1 aerobic blood culture bottle (length of incubation set at 28 days (the normal length of incubation for a fungal culture)
- Transfer 0.5 ml of blood to a MYCO bottle (to which 1.0 ml of Growth Supplement has been added - incubation preset at 42 days)
- Centrifuge the remaining blood at 3800 rpm for 15 minutes and plate the sediment to an enriched, non inhibitory fungal media (i.e. BHI w/10% sheep blood) and incubate at 30ºC for 6 weeks (total incubation time of the MYCO bottle)

The combination of these should give the fastest and least labor intensive recovery of fungi. Adding a culture of the sediment should enable the detection of molds and or yeasts that do not grow at the standard blood culture temperature of 35ºC and/or those that do not produce or consume enough gas to be detected by the analyzer.

Unlike the BD BACTEC® FX® and bioMerieux BacT/Alert®, the VersaTREK Microbial Detection System detects a change in the pressure of the headspace of the blood culture and/or MYCO bottle. This allows for the detection of any gas production and gas consumption. The BACTEC FX and BacT/Alert use sensors to detect CO_2 production only.[3] VersaTREK users oftentimes realize a faster time to detection of many species of bacteria, yeast and molds compared to competitive systems due to the detection of gas consumption.

We will continue to collect data from patient specimens as this process is put in place. During the 17 (or so) years that we have had the VersaTREK analyzers (originally known as ESP by Difco) we have never recovered a fungal isolate from the currently used Septichek system (discontinued product of BD) that was not recovered by the TREK system.



Candida guillermondii. *Candida glabrata.*

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