

Evaluation of the BDProbeTec™ ET Oven to Render Microorganisms Nonviable Prior to Testing on the BDProbeTec™ ET System

D. M. WOLFE, D. D. SHANK AND O. J. LLORIN

BD Biosciences • 7 Loveton Circle • Sparks, MD, USA 21152

ABSTRACT

■ An important criterion in the preparation of samples for any nucleic acid amplification assay is protection of laboratory personnel from exposure to potentially infectious clinical specimens. The BDProbeTec™ ET Oven,* a component of the BDProbeTec™ ET System for the detection of mycobacteria,* is designed to render microorganisms nonviable after completing the processing cycle. The BDProbeTec™ ET Oven processing cycle can heat up to forty-eight samples at $105\pm 5^\circ\text{C}$ for 30 minutes in an enclosed system. In this two-part evaluation, the BDProbeTec™ ET Oven was initially tested as a prototype at the lower end of the temperature set point ($101\pm 2^\circ\text{C}$).¹ Suspensions of 26 mycobacteria, 12 non-mycobacteria, and two yeasts, representing microorganisms that may be encountered in respiratory tract infections, were evaluated across three ovens. In the second test, the final BDProbeTec™ ET Oven was tested at the final temperature set point of $105\pm 5^\circ\text{C}$. Suspensions of *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. kansasii*, and *M. thermoresistibile* were evaluated across five ovens. The organism suspensions were spiked into pooled NALC/NaOH digested and decontaminated sputa and processed as they would be in routine mycobacterial testing on the BDProbeTec™ ET System. In all tests, each organism was prepared to a McFarland #1 standard turbidity, simulating high organism load. Each suspension was inoculated onto appropriate plated media prior to processing in the BDProbeTec™ ET Oven to verify viability. After processing in the BDProbeTec™ ET Oven, each sample was inoculated onto appropriate plated media to monitor for growth/no growth. All test organisms were viable before processing in the BDProbeTec™ ET Oven and no test organisms were viable after processing in the BDProbeTec™ ET Oven. The BDProbeTec™ ET Oven was shown to be an effective means of rendering microorganisms nonviable prior to testing on the BDProbeTec™ ET System.

BD ProbeTec™ ET



INTRODUCTION

It has been demonstrated that certain methods of heating may not render samples nonviable for safe handling by laboratory personnel. Mycobacteria can survive exposure times of up to 40 minutes in a 95°C dry-heat block.² A major contributor to dry-heat block survival is non-uniform internal temperature distribution within a sample tube, especially in the condensate that forms in the cap.

The BDProbeTec™ ET Oven is designed for operator safety and is an important component of the BDProbeTec™ ET System for the detection of mycobacteria. The BDProbeTec™ ET Oven utilizes a forced-air design to ensure uniform heating to all areas of the sample tube, including the cap.³ It can hold up to forty-eight 2 mL sample tubes. The oven cycle processes the tubes at $105\pm 5^\circ\text{C}$ for 30 minutes in an enclosed system.

The goal of this study was to validate, across several instruments and at temperatures at or below normal set point, the ability of the BDProbeTec™ ET Oven to render select microorganisms nonviable.

METHODS

In the first evaluation, three prototype BDProbeTec™ ET Ovens were evaluated at the lower end ($101\pm 2^\circ\text{C}$) of the final set point $105\pm 5^\circ\text{C}$. A panel of 40 organisms (Table 1) consisting of mycobacteria, non-mycobacteria and yeasts, that may be encountered in respiratory tract infections, were used to challenge the three instruments.

*Not available in the United States.

Table 1. Organism panel for prototype BDProbeTec™ ET Oven testing

ORGANISM	STRAIN	ORGANISM	STRAIN
<i>Candida albicans</i>	ATCC 44808	<i>Mycobacterium intracellulare</i>	ATCC 13950
<i>Cryptococcus neoformans</i>	ATCC 32045	<i>Mycobacterium kansasii</i>	ATCC 12478
<i>Enterobacter aerogenes</i>	ATCC 13048	<i>Mycobacterium malmoeense</i>	ATCC 29571
<i>Escherichia coli</i>	ATCC 11775	<i>Mycobacterium marinum</i>	ATCC 927
<i>Haemophilus influenzae</i>	ATCC 33533	<i>Mycobacterium microti</i>	ATCC 11152
<i>Klebsiella pneumoniae</i>	ATCC 13883	<i>Mycobacterium paratuberculosis</i>	ATCC 19698
<i>Legionella pneumophila</i> ss. <i>pneumophila</i>	ATCC 33152	<i>Mycobacterium scrofulaceum</i>	ATCC 19981
<i>Mycobacterium tuberculosis</i> H37Rv	ATCC 27294	<i>Mycobacterium simiae</i>	ATCC 25275
<i>Mycobacterium africanum</i>	ATCC 25420	<i>Mycobacterium szulgai</i>	ATCC 35799
<i>Mycobacterium asiaticum</i>	ATCC 25276	<i>Mycobacterium terrae</i>	ATCC 15755
<i>Mycobacterium avium</i>	ATCC 25291	<i>Mycobacterium thermoresistibile</i>	ATCC 19527
<i>Mycobacterium bovis</i>	ATCC 27290	<i>Mycobacterium triviale</i>	ATCC 23292
<i>Mycobacterium bovis</i> BCG	ATCC 35734	<i>Mycobacterium ulcerans</i>	ATCC 19423
<i>Mycobacterium celatum</i>	ATCC 51131	<i>Nocardia asteroides</i>	ATCC 3308
<i>Mycobacterium chelonae</i>	ATCC 19977	<i>Proteus vulgaris</i>	ATCC 13315
<i>Mycobacterium fortuitum</i>	ATCC 6841	<i>Pseudomonas aeruginosa</i>	ATCC 27853
<i>Mycobacterium flavescens</i>	ATCC 14474	<i>Staphylococcus aureus</i>	ATCC 25923
<i>Mycobacterium gastri</i>	ATCC 15754	<i>Staphylococcus epidermidis</i>	ATCC E155
<i>Mycobacterium gordonae</i>	ATCC 14470	<i>Streptococcus pneumoniae</i>	ATCC 6303
<i>Mycobacterium haemophilum</i>	ATCC 43160	<i>Streptococcus pyogenes</i> , group A	ATCC 16915

The organisms were grown and standardized to a McFarland #1 standard turbidity to simulate a high organism load sample. Each organism was tested in triplicate. From each suspension, 250µL was transferred to 2 mL sample tubes and washed twice with a phosphate buffered solution and resuspended to 1 mL in the same solution. From each sample tube, 100µL was inoculated onto Middlebrook 7H11 Agar, Trypticase™ Soy Agar with 5% sheep blood (BD Biosciences), Buffered Yeast Charcoal Extract Agar, or Chocolate II Agar as appropriate. Each organism replicate was placed into random locations within separate ovens and heated to 101±2°C for 30 minutes. The samples were removed from the ovens, spun in a centrifuge to collect condensate in the tubes, and 100µL of each sample was inoculated onto plated media. All media were incubated at appropriate temperatures for up to eight weeks. Observations were performed weekly. If no growth of the test organism was seen at eight weeks, the organism was considered nonviable.

In the second evaluation, five BDProbeTec™ ET Ovens were tested at the final temperature set point of 105±5°C with mycobacterial suspensions (Table 2). Suspensions were prepared to a McFarland #1 standard turbidity, simulating a high organism load sample, in Wash Buffer 1 (this reagent is part of the Specimen Processing Kit for both the *Mycobacterium tuberculosis* Complex Direct Detection* and Mycobacteria Culture Identification* Assays on the BDProbeTec™ ET System). Colony counts were performed on each suspension using Middlebrook 7H11 Agar plates.

Table 2. Organism panel for final BDProbeTec™ ET Oven testing *M. tuberculosis*.

ORGANISM	STRAIN	CFU/mL
<i>M. tuberculosis</i>	ATCC 27294	7.4x10 ⁶
<i>M. avium</i>	ATCC 25291	3.7x10 ⁷
<i>M. intracellulare</i>	ATCC 13950	5.3x10 ⁶
<i>M. kansasii</i>	ATCC 12478	5.2x10 ⁷
<i>M. thermoresistibile</i>	ATCC 19527	7.6x10 ⁶

REFERENCES

1. D. M. Wolfe, D. D. Shank, and O. J. Llorin. 1997. Evaluation of the BD Lysolyzer to Render Microorganisms Nonviable Prior to SDA. Abstr. U-053 p. 553. Abstr. 97th Gen. Meet. Am. Soc. Microbiol.
2. Zwadyk, P.J.R., J.A. Down, N. Myers, and M.S. Dey. 1994. Rendering mycobacteria safe for molecular diagnostic studies and development of a lysis method for strand displacement amplification and PCR. J. Clin. Microbiol. 32:2140-2146.
3. Reichler et al. "Forced Hot Air Heating Device", US Patent No. 5,783,439 issued July 21, 1998.

To simulate an actual sample, 500µL of pooled NALC/NaOH processed sputa was added to all 2 mL sample tubes containing 1 mL of each organism suspension. The sample tubes were then vortexed, centrifuged, and the supernatant decanted, according to the BDProbeTec™ ET mycobacterial assay procedure. Three separate oven cycles were performed using nine replicates of each organism across the five ovens (n=135). All sample tubes were placed in random locations within the ovens and heated to 105±5°C for 30 minutes. After oven processing, the tubes were centrifuged to collect condensate in the tube and each pellet was assessed for viability by plating the entire pellet onto an appropriately labeled Middlebrook 7H11 Agar plate. All plates were incubated at 37±1°C in 5% CO₂ for eight weeks. If no growth of the test organism was seen within the eight-week incubation period, the microorganism was considered nonviable.

RESULTS

In the first test that evaluated three prototype BDProbeTec™ ET Ovens at the low end temperature set point, none of the 40 organisms (tested in triplicate) grew after eight weeks of incubation. All of the organisms showed viability prior to oven processing.

In the second test that evaluated five BDProbeTec™ ET Ovens at the final temperature set point, none of the 135 replicates of the five mycobacterial species tested showed growth after eight weeks of incubation.

DISCUSSION

The results of the extensive testing performed demonstrate that the BDProbeTec™ ET Oven is effective in rendering microorganisms nonviable that may be encountered in clinical respiratory samples as well as the infectious pathogens specifically tested for by the *Mycobacterium tuberculosis* Complex Direct Detection and Mycobacteria Culture Identification Assays. The mycobacterial sample processing method for the BDProbeTec™ ET System was developed with safety and ease of workflow strongly in mind. Mycobacterial decontamination at an early step in the procedure allows subsequent manipulations of samples for probe testing to be performed at the laboratory bench, free from the constraints of a biosafety cabinet.

The BDProbeTec™ ET Oven has several features that are designed to provide added safety benefits. The processing cycle will not initiate until a self-locking mechanism is activated by complete door closure. When closed, a gasket lining the BDProbeTec™ ET Oven door provides an air-tight seal. Instrument temperature is monitored throughout the processing cycle to insure that the set point temperature is reached within a specified time. If the instrument detects temperatures out of specification, the user is notified through an interactive display panel. Once the processing cycle is completed, the locking mechanism will not allow the door to be reopened until the instrument temperature cools to 50°C. Any air evacuated from the instrument during the processing cycle must first pass through a High Efficiency Particle Arresting (HEPA) filter.

CONCLUSION

- The enclosed forced-air design of the BDProbeTec™ ET Oven ensures that all sample tubes are subjected to uniform heating. As a result, microorganisms are rendered nonviable after appropriate incubation.
- This step in the BDProbeTec™ ET mycobacteria sample processing, in turn, promotes safety and ease of workflow in the laboratory.