

## Evaluation of a Novel Specimen Transport System (Venturi Transystem) for Anaerobic Bacteria

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The Venturi Transystem (Copan Diagnostics, Corona, CA), with and without charcoal, is designed for transport of clinical specimens. It was evaluated for its ability to maintain the viability of pure cultures of selected anaerobic bacteria. Results indicated that the system supported survival of test strains within the time frame that most clinical specimens require to reach the clinical laboratory.

Protection of anaerobic bacteria from exposure to oxygen during the transport of clinical specimens to the laboratory is important for the survival of these organisms. Although some transport systems have been tested and are effective in maintaining viability of anaerobes [1 – 4], they are costly and some are breakable. The Venturi Transystem (Copan Diagnostics, Corona, CA) is nonbreakable and inexpensive and offers an alternative to other systems for the transport of clinical specimens obtained from suspected anaerobic infections.

We tested five reference strains (*Bacteroides fragilis* American Type Culture Collection [ATCC] 25285, *Bacteroides thetaiotaomicron* ATCC 29741, *Clostridium perfringens* ATCC 13124, *Fusobacterium nucleatum* ATCC 25586, and *Peptostreptococcus anaerobius* ATCC 27337) and five clinical isolates (*Prevotella bivia*, *Fusobacterium necrophorum*, *Peptostreptococcus anaerobius*, *Fusobacterium nucleatum*, and *Clostridium difficile*). These isolates were stored in 20% skim milk at -70°C until use. They were taken from frozed stock and transferred twice on Brucella blood agar (BBA) supplemented with vitamin K<sub>1</sub> and hemin, and they were incubated at 37°C for 48 hours in an anaerobic chamber (Anaerobe Systems, San Jose, CA).

The Venturi Transystem used for this study consisted of a plastic transport tube containing Amies medium either with or without charcoal. At the time of manufacture, the plastic sheath is pinched above the solid medium to prevent spillage and to reduce the surface area for oxygen diffusion. The tube is flushed with nitrogen gas just before it is sealed with a plastic cap. The tube and a swab are packaged in a gas-impermeable plastic wrap that is flushed with nitrogen to provide an anaerobic environment. The atmosphere of the tube and swab are anaerobic before being opened. Both formulations of the medium, with charcoal and without charcoal, were evaluated and compared with the BBL Port-A-Cul transport tube (Becton Dickinson Microbiology Systems, Cockeysville, MD).

All test strains except for *Clostridium* species were inoculated into Brucella broth to achieve a density approximately equal to the no. 4 McFarland standard; a no. 5 McFarland standard was used for the *Clostridium* species, which have large cells. Colony counts of the inocula were verified by quantitative plating; in this procedure, two serial dilutions of 1:100 were prepared, and then quantitative loops of 0.01 and 0.001 mL were used for plating onto BBA.

To inoculate the swabs, the seal of the plastic tube was broken by removing the plastic cap, exposing the contents of the tube to atmospheric conditions. Duplicate swabs, using each transport system and each type of bacteria, were inoculated with 0.1 mL of this cell suspension and placed in ambient air for 0, 4, 24, and 48 hours, for a total of 240 swabs. At the specific time points, the swabs were removed and placed into 10 mL of tryptic soy broth to resuspend the bacteria. Quantitative loops of 0.01 and 0.001 mL were used for plating onto BBA.

After the BBA plates were incubated for 48–72 hours, the colonies were counted. The colony counts from the duplicate swabs were averaged. The colony counts of test strains that survived in the transporter at 4, 24, and 48 hours were compared with colony counts determined at the time of inoculation (0 hours).

All three transporter systems supported viability of the anaerobes tested at 4 hours and over a 24-hour period (table 1). At 4 hours there was an insignificant loss of viability (data not shown) compared with the 0 hour control, while at 24 hours, only a 1–2 log<sub>10</sub> loss of some organisms in some of the systems was observed. The concentration of all strains was at least 3.5 x 10<sup>4</sup> organisms/mL. This concentration is sufficient to achieve a number of organisms that is considered acceptable for obtaining good growth from a swab of a clinical specimen since ~ 10<sup>5</sup> cfu/mL generally yields 4+ growth on a streak plate [5]. The ATCC strains *B. fragilis* and *B. thetaiotaomicron* survived equally well for up to 48 hours in all three transporters. In all three transporters, the ATCC strains *F. nucleatum* and *P. anaerobius* survived for up to 48 hours with only ≤ 1 log<sub>10</sub> loss of viability compared with the 0 time assay. Overall, viability of the clinical isolates was not maintained as well as the ATCC strains in any of the transporters.

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Table 1. Survival of anaerobes in the Venturi Transystem and the Port-A-Cul transport tube.

Type of strain	Concentration in organisms per swab after 0, 24, and 48 hours								
	Amies media with charcoal			Amies media without charcoal			Port-A-Cul		
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
<b>ATCC</b>									
<i>B. fragilis</i>	5 x 10 <sup>7</sup>	3 x 10 <sup>7</sup>	2 x 10 <sup>7</sup>	5 x 10 <sup>7</sup>	4 x 10 <sup>7</sup>	3 x 10 <sup>7</sup>	1 x 10 <sup>7</sup>	1 x 10 <sup>7</sup>	1 x 10 <sup>7</sup>
<i>B. thetaiotaomicron</i>	3 x 10 <sup>7</sup>	3 x 10 <sup>7</sup>	2 x 10 <sup>7</sup>	3 x 10 <sup>7</sup>	3 x 10 <sup>7</sup>	3 x 10 <sup>7</sup>	4 x 10 <sup>7</sup>	3 x 10 <sup>7</sup>	3 x 10 <sup>7</sup>
<i>C. perfringens</i>	3 x 10 <sup>6</sup>	2 x 10 <sup>6</sup>	6 x 10 <sup>5</sup>	3 x 10 <sup>7</sup>	6 x 10 <sup>6</sup>	1 x 10 <sup>6</sup>	1 x 10 <sup>7</sup>	1 x 10 <sup>7</sup>	1 x 10 <sup>7</sup>
<i>F. nucleatum</i>	2 x 10 <sup>7</sup>	5 x 10 <sup>6</sup>	2 x 10 <sup>6</sup>	2 x 10 <sup>7</sup>	1 x 10 <sup>7</sup>	5 x 10 <sup>6</sup>	3 x 10 <sup>7</sup>	6 x 10 <sup>6</sup>	7 x 10 <sup>6</sup>
<i>P. anaerobius</i>	2 x 10 <sup>6</sup>	9 x 10 <sup>5</sup>	5 x 10 <sup>5</sup>	2 x 10 <sup>6</sup>	2 x 10 <sup>6</sup>	3 x 10 <sup>5</sup>	9 x 10 <sup>6</sup>	4 x 10 <sup>6</sup>	4 x 10 <sup>6</sup>
<b>Clinical</b>									
<i>P. bivia</i>	4 x 10 <sup>7</sup>	5 x 10 <sup>5</sup>	0	4 x 10 <sup>7</sup>	4 x 10 <sup>6</sup>	1 x 10 <sup>4</sup>	5 x 10 <sup>7</sup>	5 x 10 <sup>7</sup>	2 x 10 <sup>7</sup>
<i>P. anaerobius</i>	1 x 10 <sup>7</sup>	5 x 10 <sup>5</sup>	0	1 x 10 <sup>7</sup>	3 x 10 <sup>6</sup>	2 x 10 <sup>6</sup>	1 x 10 <sup>7</sup>	5 x 10 <sup>5</sup>	4 x 10 <sup>5</sup>
<i>F. nucleatum</i>	8 x 10 <sup>6</sup>	5 x 10 <sup>4</sup>	0	8 x 10 <sup>6</sup>	4 x 10 <sup>4</sup>	5 x 10 <sup>4</sup>	2 x 10 <sup>7</sup>	4 x 10 <sup>6</sup>	4 x 10 <sup>6</sup>
<i>F. necrophorum</i>	2 x 10 <sup>7</sup>	2 x 10 <sup>7</sup>	1 x 10 <sup>5</sup>	2 x 10 <sup>7</sup>	2 x 10 <sup>6</sup>	1 x 10 <sup>6</sup>	2 x 10 <sup>7</sup>	3 x 10 <sup>6</sup>	3 x 10 <sup>6</sup>
<i>C. difficile</i>	4 x 10 <sup>6</sup>	1 x 10 <sup>5</sup>	2 x 10 <sup>7</sup>	5 x 10 <sup>6</sup>	1 x 10 <sup>5</sup>	3 x 10 <sup>5</sup>	1 x 10 <sup>6</sup>	2 x 10 <sup>6</sup>	5 x 10 <sup>6</sup>

NOTE: ATCC= American Type Culture Collection.



Figure 1: The Venturi Transystem swabs with charcoal (left) and without charcoal (right).

The Venturi Transystem containing Amies medium without charcoal sustained viability for all test strains up to 48 hours, and the number of viable organisms was comparable with the Port-A-Cul system (table 1). The Venturi Transystem containing Amies medium with charcoal maintained viability of all test strains for 24 hours. However, clinical strains of *P. bivia*, *P. anaerobius*, and *F. nucleatum* did not survive after 48 hours. Although the five ATCC strains and two of the five clinical isolates, *C. difficile* and *F. necrophorum*, survived well in the Amies media with charcoal, in general this medium did not sustain viability as well as the other media (table 1).

Both of the Venturi Transystem media sustained viability of all the strains tested within the time frame that most clinical

specimens require to reach the laboratory, i.e., <24 hours. Overall, the Venturi Transystem compared favorably with the Port-A-Cul system, and since the cost of the Venturi Transystem is about one-third of that of the Port-A-Cul system, it can provide a cost-effective alternative for the local transport of anaerobic bacterial specimens to the clinical laboratory. Further studies to evaluate anaerobes from clinical specimens with use of the Venturi Transystem are planned.

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