

Improved Procedure for Transport of Dental Plaque Samples and Other Clinical Specimens Containing Anaerobic Bacteria

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An improved transport system for samples containing anaerobic bacteria was developed. This system increased the recovery rate of anaerobic bacteria up to 28.8% as compared to a commonly used method.

Because much of the oral flora is comprised of anaerobic microorganisms (5, 7), techniques for collection and transport of oral specimens should maintain anaerobiosis. Current techniques frequently overlook the requirements for anaerobic transport. This note describes a dual-tube system (DT) designed to improve anaerobic transport of dental plaque specimens and other clinical specimens containing anaerobic bacteria.

(This paper is based on a portion of a thesis submitted by C.A.S. to the graduate school, University of Maryland at Baltimore, in partial fulfillment of the requirements for the Ph.D. degree.)

The outer chamber of the DT consisted of an test tube (18 by 142 mm) and a no. 1 butyl rubber stopper (Bellco Glass, Inc.). The inner tube was a 1-dram (ca. 3.7-ml) screw-capped vial (without the cap) which rested on glass beads in the outer chamber. The glass beads served to raise the inner vial to near the orifice of the outer tube so that sonic oscillation of the sample with the short probe of the Kontes ultrasonic cell disruptor (Kontes Biomedical Products, Vineland, N.J.) could be performed. The outer tube was capped with a size 20A Morton closure without fingers (Bellco Glass, Inc.) (Fig. 1a), and the assembly was autoclaved along with a glass beaker containing butyl rubber stoppers and a flask of reduced transport fluid (6) containing 0.0001% resazurin and 0.05% cysteine-hydrochloride (in water) in place of dithiothreitol. The sterile materials were then passed into an anaerobic glove box (1) (Coy Laboratory Products, Inc., Ann Arbor, Mich.) containing oxygen-free gas (OFG; 85% nitrogen, 10% hydrogen, 5% carbon dioxide). Before the plaque sampling procedure, the desired volume of transport fluid was pipetted into the inner vial, the chamber was sealed aseptically with a rubber stopper, using a hemostat and tightening by hand (Fig. 1b), and

the Morton closure was replaced. When closed, there must be a space between the bottom of the stopper and the top of the vial to allow an airtight seal. The DT was removed from the glove box immediately before sample collection.

The dental plaque specimen was collected from a gingival crevice, using a sterile periodontal explorer under OFG flow. The OFG was directed on the sampling site by means of dual metal cannulas connected to the gas tank by rubber tubing. As the sample was placed into the transport fluid, the gas was directed into the inner vial of the DT. The gas assembly was then quickly fixed to a ring stand, and the rubber stopper was held in the orifice of the outer chamber with a bent hemostat (Fig. 2). Oxygen was flushed from the mouth of the tube for several seconds before the cannulas were removed from the DT, and the rubber stopper was tightened by hand. The assembly was placed in a press (Bellco Glass, Inc.) and immediately introduced into the anaerobic glove box. During the entire procedure the resazurin remained colorless.

A subgingival plaque sample, collected as described above, and log phase cultures of oral isolates of *Bacteroides melaninogenicus* subsp. *intermedius* and *Peptostreptococcus micros*, grown in supplemented brain heart infusion (BHI) broth (3), were used to test the effectiveness of this system. The log phase cultures were diluted in reduced transport fluid so that the number of organisms approximated the number collected in the dental plaque samples. Each of these three specimens was then sonically disrupted, diluted, and plated in triplicate on MM10 sucrose blood agar (6) to determine the maximum recovery of the bacteria in each sample. Two equal-sized portions of each of the original samples were then removed to test the transport systems. One aliquot was placed into a DT and the other into a 1-dram vial (screw-capped tube [SCT]) which was enclosed in a GasPak jar (Baltimore Biological Laboratory;

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TABLE 1. Effect of anaerobic transport on the colony-forming units (CFU) recovered from subgingival dental plaque and pure cultures of anaerobic bacteria

Culture	CFU		% Decrease in CFU ^a		
	Original sample	After transport experiment		SCT	DT
		SCT	DT		
<i>B. melaninogenicus</i> subsp. <i>intermedius</i>	2.67×10^7	6.00×10^6	1.34×10^7	77.5	49.8
<i>P. micros</i>	1.41×10^7	5.87×10^6	7.03×10^6	58.4	50.1
Subgingival plaque sample	4.63×10^5	2.45×10^5	3.80×10^5	46.7	17.9

^a Each value indicates [(CFU of original sample - CFU after transport) (100)]/(CFU of original sample).

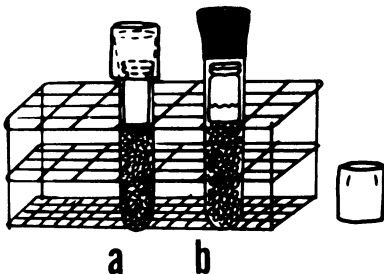


FIG. 1. (a) DT transport system before the addition of transport fluid to the inner vial. (b) DT system after addition of transport fluid to the inner vial.

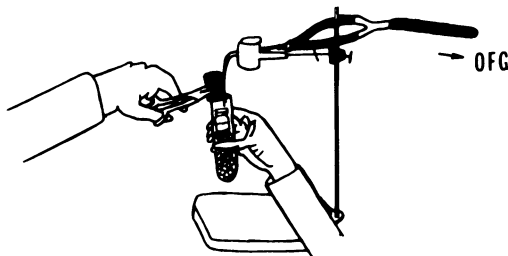


FIG. 2. Closure of the DT system after sampling.

Cockeysville, Md.). Both transport systems were taken to the dental clinic and handled as if samples were being taken. The sample in the SCT was flushed with OFG during the simulated inoculation and closure, and was sealed with parafilm for transport to the laboratory. Simulated inoculation of the DT was performed as explained above. Both samples were again diluted and plated in the anaerobic glove box, and recoveries were compared to those of the original samples.

After 10 days of incubation at 36°C, total viable and differential colony counts were performed on the three sets of plates. Each colony type recovered from the plaque sample was subcultured to check for purity, and a smear of each isolate was stained by the Kopeloff modification

TABLE 2. Recovery of various types of bacteria from a dental plaque sample transported by SCT and DT systems

Sample	% Recovery ^a				
	Gram-positive rods	Gram-negative rods	Gram-positive cocci	Unidentified	Anaerobic gram-positive cocci
Original	26.8	55.3	13.1	4.8	9.4
After transport in SCT	46.7	42.5	10.7	0	2.9
After transport in DT	35.1	55.1	9.8	0	8.0

^a Values indicate percentage of total CFU.

of the Gram stain (3), using a basic fuchsin counterstain. Gram-positive cocci were again subcultured to determine oxygen sensitivity.

The loss in recovery of the dental plaque bacteria and the pure cultures of anaerobes as determined from the colony-forming units of original and transported samples were compared. The percent recovery of the three specimens transported in the SCT system decreased considerably more than those transported in the DT system, indicating a higher level of oxygen contamination in the SCT. As compared to the SCT, the DT improved recovery of plaque bacteria by 28.8%, *P. micros* by 8.3%, and *B. melaninogenicus* by 27.7% (Table 1). When the percent recoveries of various types of bacteria in the plaque specimen were examined, the recoveries of Gram-negative rods and anaerobic Gram-positive cocci were found to be higher after transport by the DT versus the SCT system (Table 2).

During cultural studies of the subgingival dental plaque flora, several investigators did not protect the collection vial from oxygen contamination during transport of the specimen to the laboratory (2, 4-7). Few investigators have even

used the protection afforded by the SCT described above. The DT offers protection of the collection medium against oxidation, as evidenced by the failure of the resazurin indicator in the transport fluid to turn from colorless to pink, and was shown to greatly improve the recovery of a microbial specimen containing a high percentage of anaerobes as well as pure cultures of anaerobes. The method should be useful in cultural studies of dental plaque and other clinical specimens containing anaerobic bacteria.

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