Anaerobic specimen transport system

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Summary. Degassed dimethyl silicone oil may be used as a transport medium for specimens containing anaerobic microorganisms.

Successful isolation of oxygen-sensitive anaerobic bacteria from clinical or environmental specimens requires anaerobic transportation of the specimen from collection site to laboratory. Methods used to obtain anaerobiosis include direct inoculation into pre-reduced media, immersion in oxygen-free gas, or catalytic oxygen reduction[1,2]. We have found that dimethyl silicone oil free of dissolved oxygen may be used as a medium for transporting oxygen-sensitive microbes.

Dimethyl silicone oil is a biologically inert, thermally stable fluid of density 0.97 g/ml. The solubility of oxygen in the oil is 26 ml O_2/dl, which is approximately 8 times that in water (3.16 ml O_2/dl). An oxygen electrode was used to determine the oxygen activity in water and dimethyl silicone oil at a variety of oxygen partial pressures. The equilibrium oxygen activities in a 2-phase water/oil system were identical in each phase. Since oxygen activity is the product of concentration and an activity coefficient, it follows that the activity coefficient of oxygen dissolved in dimethyl silicone oil is approximately $\frac{1}{8}$ that in water. Deoxygenated dimethyl silicone oil in contact with an aqueous phase will act as an 'oxygen sink' causing a decrease in the aqueous oxygen activity and concentration.

The anaerobe *Clostridium innocuum* ATCC 14501 was used to test this concept. Approximately 20 ml dimethyl silicone oil, with a viscosity of 1000 centistokes (Dow Corning 200 Fluid) was degassed and sterilized by evacuation at 130 °C for 4 h. Cultures were grown in thioglycollate broth medium and viable counts were made using pre-reduced thioglycollate agar in bottles gassed with anaerobic-grade CO_2. Bacterial cultures were diluted 1:10 with aerated nutrient broth and held for 5 min to simulate exposure to air during specimen collection. 2 ml samples were then incubated for 24 h at 23 °C in an empty tube or immersed in degassed dimethyl silicone oil. The table shows that immersion of samples in degassed silicone oil prevented a decline in viable counts.

Effect of degassed dimethyl silicone oil on survival of *Clostridium innocuum* ATCC 14501

	CFU/ml* (average of duplicates)
Original suspension in nutrient broth	5.8×10^4
Suspension under degassed silicone oil 24 h	6.8×10^4
Suspension exposed to air 24 h	9.5×10^2

* Colony forming units/ml.

The separation of dimethyl silicone oil from specimens was studied using guinea-pig tissues. 1 g samples of blood, kidney, muscle, liver or mesentery tissues, and swabs of intestinal contents were immersed in the oil. All tissues sank to the bottom of the tubes, consistent with the relative densities of oil and tissue. Tissues were removed with forceps and placed in nutrient broth. Gentle shaking caused the tissues to sink into the aqueous medium leaving a thin layer of oil on the surface. Oil also quickly separated from specimens when drops of blood and swabs were placed in the broth. Normal procedures may be used to Gram stain broth cultures after immersion in oil. Gram stains may be made on tissues or swabs after immersion in oil if care is taken to rub or roll the tissue or swab on the slide, in order to ensure the slide is wet.

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2 T.D. Wilkins and F. Jimenez-Ulate, J. clin. Microbiol. *2*, 441 (1975).

The mutagenic mode of action of benzimidazole

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Summary. By using a bacterial mutant strain with a known restricted reversion pattern, it has been shown that benzimidazole induces an unidirectional base change also in vivo. The in vitro demonstrated strict G → A transition has thus been confirmed.

Benzimidazole has been shown to induce mutations of the base substitution type in *Salmonella typhimurium*[2]. This behaviour could have been expected because of the purine-like structure of this compound. It could then be demonstrated, that indeed benzimidazole is incorporated into the nucleic acids of *Escherichia coli*[3,4]. An investigation into the possible mode of action yielded the somewhat unexpected result that benzimidazole is a very specific mutagenic agent; it had be concluded from in vitro results on the transcriptional use of benzimidazole nucleotide and on the translation of benzimidazole containing codons that this compound induced strictly G → A transitions in this direction only[5]. In view of the theoretical importance of such a mode of action it seemed indicated to test this conclusion by another way, if at all possible in an in vivo assay. One possibility would have been to investigate the reversion characteristics of benzimidazole induced mutants: such mutants should not be reverted by benzimidazole itself nor by hydroxylamine, which has a similar specificity. A much less tedious procedure, however, was found by using bacterial mutants whose reversion patterns in terms of possible base changes are already known. In its simplest application it should only be necessary to perform a mutagenicity test with such a mutant in order to obtain a clearcut 'yes' or 'no'