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Butyl Rubber Stoppers Increase the Shelf Life of Prereduced, Anaerobically Sterilized Media

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Butyl rubber stoppers as compared with neoprene or black rubber stoppers significantly increased the shelf life of prereduced, anaerobically sterilized media for growth of obligately anaerobic bacteria.

Hungate et al. (4) measured the permeability of butyl and common rubber stoppers to oxygen, carbon dioxide, and hydrogen. Due to the very low permeability of these gases through butyl rubber stoppers (e.g., <0.025 μl of O_2 per ml of gas in 10 days), butyl rubber appeared to be superior to common rubber for stoppers; however, no information on the shelf life of prereduced, anaerobically sterilized (PRAS) media (5) made with butyl stoppers was reported.

This study was suggested when 50 tubes of PRAS media prepared two years earlier were observed. The tubes of media had been prepared in 1971 in pyrex tubes (14 by 100 mm). Some of these had been stoppered with no. 00 common black rubber stoppers; others had been stoppered with no. 00 neoprene rubber stoppers, and still others had been stoppered with no. 00 butyl rubber stoppers. In 1973 we found that all of the tubes with neoprene and black rubber stoppers had oxidized, whereas none of the tubes stoppered with the butyl stoppers had oxidized.

We then tested the hypothesis that use of butyl rubber stoppers increases the shelf life of PRAS media. Number 1 size stoppers were obtained as follows: A. H. Thomas Co. butyl rubber stoppers no. 1, catalog no. 8748-B21; Bellco Glass, Inc., closures, stopper, Black Rubber no. 1, catalog no. 1926-00001; Fisher Scientific Co. rubber stopper no. 1, catalog no. 14-130C; Fisher Scientific Co. rubber stopper no. 1, Neogrene, catalog no. 14-141B. Culture tubes were Bellco anaerobic culture tubes, size 18 by 142 mm, catalog no. 2046-18142.

The stoppers were notched to identify each brand, autoclaved in a solution of alkaline laboratory detergent, and thoroughly rinsed in warm water. They were randomly mixed. A single batch of PY medium was tubed in clean 18-mm Bellco tubes under an oxygen-free nitrogen atmosphere and closed with the stoppers (3). Stoppers were tightly seated, clamped (2), and sterilized in the autoclave. Tubes were marked with date-coded quality control numbers and stored at room temperature in a dark cabinet.

At intervals, the tubes were removed from the cabinet and shaken 50 times to equilibrate the tube atmosphere with the PY medium. Oxidized tubes, as evidenced by the pink color of resazurin, were counted and recorded. At the end of the experiment >10% of all nonoxidized tubes were opened and exposed to air to ensure that the resazurin indicator was still functional (it was).

TABLE 1. *Shelf life of PRAS media using various stoppers*

Tubes oxidized at (month):	No. of tubes prepared with stoppers of:			
	Butyl (A. H. Thomas)	Black rubber (Bellco)	Neoprene (Neogrene)	Black rubber (Fisher)
Expt 1 (total)	31	30	ND[a]	30
1	0	0		0
2	0	5		26
3	0	12		30
6	0	21		30
12	6	29		30
20	10	30		30
Expt 2 (total)	50	49	49	80
1	0	0	0	0
2	0	0	0	67
3	0	4	0	78
6	0	12	9	80
13	0	46	46	80

[a] ND, Not done.

Clostridium novyi type B, Virginia Polytechnic Institute no. 8099, carried in CM medium (3), was used to test media for growth suitability. One drop of a 24-h culture was used to inoculate each tube by the method of Virginia Polytechnic Institute and State University Anaerobe Laboratory (3).

Table 1 shows the results of the two experiments. Seventeen of eighteen (94%) of the unoxidized tubes closed with the butyl rubber stopper that were inoculated with *C. novyi* type B showed growth. No growth was obtained in any of the inoculated oxidized tubes, although growth was seen in one slightly oxidized tube that was inoculated. Two unoxidized tubes of media under Neogrene stoppers would not support growth.

There are disadvantages in the routine use of the brand of butyl rubber stoppers used. This stopper has a recessed top extending halfway through the stopper. This, plus the pliability of the stopper, requires seating the stopper quite deeply into the neck of the tube to obtain a proper seal. Thus, the top of the stopper is difficult to grasp for removal. Stoppers of this same soft butyl rubber composition, but completely solid throughout, and perhaps oversized or size no. 2 stoppers would be even more successful for use in the 18-mm tapered neck tube. We also found that the no. 4 size butyl stopper shrinks appreciably when used in 25-mm tubes containing media with fatty acids (1, 3).

These butyl rubber stoppers are a distinct advantage to small laboratories using certain media only infrequently and to all laboratories storing viable cultures of obligately anaerobic bacteria or working with slow-growing, very strict anaerobes such as acetate-utilizing methanogens.

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