JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1975, p. 549-551 Vol. 2, No. 6
 Printed in U.S.A.

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Retention of Mercurial Preservatives in Desiccated Biological Products

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Received for publication 2 September 1975

A variety of bacterins, vaccines, and antisera retained greater than 90% of their original level of mercurial preservative after lyophilization, and this might influence certain uses of these products.

Biological products recommended as prophylactic or diagnostic reagents usually contain antimicrobial preservatives, and some of these products are subsequently desiccated to enhance their stability. In addition to their expected use, many biological products are used by investigators for other purposes and are often combined with preservative-sensitive components, such as tissue culture cells, live virus vaccines, embryos, etc. In previous papers (2, 3) it was reported that a large percentage of the formaldehyde and phenol preservatives was retained by desiccated biological products. For this reason it was desirable to determine how much of the mercurial preservative, thimerosal, was retained by desiccated biologics.

Thimerosal, an organic compound containing 49.55% mercury, is normally used as a preservative for biologics at concentrations of 1:5,000 to 1:20,000. Biologics preserved with thimerosal usually have a typical odor, which might imply that the thimerosal would be volatile. Several investigators have reported extensive losses of mercury from aqueous samples during storage. Lyophilization, however, has been reported (1) as a satisfactory means of storing samples to be tested for mercury contamination of animal tissues. Therefore, the following experiments were made to determine the amount of thimerosal retained in desiccated antisera, vaccines, and bacterins.

The samples were commercial biologics, which were lyophilized for this study but are not normally lyophilized for general distribution, plus several samples made to contain ingredients often found in biological products. Solutions of phosphate-buffered saline were used at three pH levels to determine the effect of pH on the retention of mercurial preservatives. Other solutions, such as thioglycolate, cysteine, and glutathione, were also tested to determine the influence of sulfur-containing compounds on the retention of mercurials. All samples were tested for mercury initially and after being treated with amounts of thimerosal equivalent to 50, 200, or 400 μg/ml.

The samples were divided, with part being retained as liquid and the remainder dispensed into vials lyophilized for about 24 h. Two vials of serum were used as controls for the lyophilization process, and after lyophilization they contained <3% moisture, as determined by the vacuum-oven technique. The desiccated samples were rehydrated by adding a volume of distilled water equivalent to the original fill-volume.

The mercury content of the samples was determined by atomic absorption spectrophotometry (4), and the mercury values were multiplied by 2.018 to convert the results to equivalent amounts of thimerosal. The atomic absorption procedure involves the digestion of the samples with H_2SO_4-HNO_3 and permanganate, followed by reduction with $SnCl_2$. The mercury was purged from the sample and swept through the absorption cell by a stream of air. Samples and standards were treated in an identical manner. This test could easily be modified for greater sensitivity, but all of these samples were assayed in the same range with only a fourfold difference in sample size.

The amount of thimerosal found in the liquid and rehydrated samples before and after treatment with added amounts of thimerosal are presented in Table 1. Replicate values were averaged, and the percent retained was calculated for each sample. There was little difference in retention characteristics among the various biological products, except for a few values that were difficult to explain. There

TABLE 1. *Amount of thimerosal found in liquid biological products and rehydrated samples to estimate the percentage of thimerosal retained during lyophilization*

Sample description	Thimerosal (μg/ml)			
	Approximate amt added	Found in liquid product	Found in rehydrated product	% Retained in desiccated product
Leptospira pomona				
Bacterin 1	0	90	91	101
	50	150	142	95
	200	270	309	114
Bacterin 2	0	328	335	102
	50	392	414	106
	400	732	593	81
Erysipelas				
Bacterin 1	0	0	2	
	50	47	39	83
	200	176	173	98
Bacterin 2	0	84	64	76
	50	122	93	76
	200	249	246	99
Wart vaccine				
1	0	0	2	
	50	42	29	69
	200	212	99	47
2	0	89	72	81
	50	138	118	86
	200	255	251	98
Mink enteritus vaccine, *Clostridium botulinum* bacterin-toxoid				
1	0	36	30	83
	50	89	77	87
	200	203	185	91
2	0	0	1	
	50	35	28	80
	200	194	183	94
C. perfringens toxoid	0	6	3	
	50	36	38	106
	200	187	170	91
Pasteurella bacterin	0	43	38	88
C. chauvoei-septicum bacterin	0	73	68	93
Tetanus toxoid	0	6	3	
	50	45	42	93
	200	177	161	91
Bacterial antiserum, bovine formula	0	81	66	81
Escherichia coli-Salmonella enteritidis-Pasteurella antiserum	0	88	75	85
	50	139	122	88
	200	257	231	90
Corynebacterium pyogenes-Pasteurella multocida				
Antiserum 1	0	97	85	88
Antiserum 2	0	91	81	89
	50	121	112	93
	200	271	243	90
PBS[a]				
pH 5.5	50	43	34	79
	200	174	171	98
pH 7.0	50	46	43	93
	200	155	176	114
pH 8.5	50	51	48	94
	200	200	210	105
Albumin, 25 mg/ml in PBS, pH 7.0	0	0	1	
	50	50	44	88
	200	194	188	97
Thioglycolate medium	50	53	41	77
	200	157	168	107
Cysteine, 0.1% in PBS	50	50	32	64
	200	177	183	103
Glutathione, 0.1% in PBS	50	40	32	80
	200	168	138	82

[a] PBS, Phosphate-buffered saline.

was apparently little influence by pH or the presence of sulfur-containing compounds on the retention of the mercurial preservative. The overall average amount of thimerosal retained by the desiccated products was 92% of the amount found in the liquid product. It is likely that the percent retained would have been slightly higher if it had been possible to accurately restore (quantum sufficient) the samples to original volume instead of adding diluent equivalent to the original fill-volume.

The data reported here and in previous reports (2, 3) indicate that a large percentage of formaldehyde, phenol, and mercurial preservatives is retained by biological products during the usual lyophilization process. These preservatives are not proportionally removed with the liquid portion of the product as might be expected. Investigators should be cautious when using preserved and lyophilized biological products in preservative-sensitive systems. Caution should also be exercised with products intended for certain immunological uses, as these preservatives evidently concentrate in the microenvironment of lyophilized antigenic material, which could denature the antigen.

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