Retention of Preservative Levels of Phenol in Desiccated Biological Products

JOHN R. PEMBERTON

Veterinary Services Laboratories, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, Ames, Iowa 50010

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Preservative levels of phenol retained by desiccated biological samples ranged from 3 to 73%, with highly proteinaceous samples retaining the most phenol.

Most bacterins, vaccines, and antisera used as prophylactic or diagnostic reagents contain antimicrobial preservatives, and some of these products are subsequently desiccated to enhance their stability. As mentioned in the companion paper (2), it is sometimes desirable to mix these desiccated biologics with products containing live organisms to form multicomponent products or to evaluate these products with test systems sensitive to preservatives. We were also interested in how well these samples were preserved. For these reasons we felt that it would be desirable to know how much preservative was retained in desiccated biologics.

Phenol is a solid at room temperature (melting point, 43 C), but under normal conditions aqueous solutions of phenol will give off the characteristic odor. Because of the unusually high concentrations of formalin previously found to be retained in desiccated biologics (2), similar experiments were designed to evaluate the retention characteristics of phenol.

The samples used in this study were commercial biologics plus phenolized solutions of phosphate-buffered saline (PBS) and albumin (90 mg/ml). The PBS was used at pH 5.5, 7.2, and 8.5 to determine the effect of pH on the retention of phenol. The PBS and albumin solutions were treated with three levels of phenol to determine if there was an influence of concentration on the retention of phenol. These samples were divided, with part being retained as liquid and the remainder dispensed in four vials which were lyophilized for about 24 h. Two additional vials containing serum were used to monitor the lyophilization process. The moisture content of these serum samples was estimated by the vacuum-oven method to be <3%.

The phenol content of the samples was determined by the reaction with 4-aminoantipyrine (3). Briefly, aliquots of diluted sample were treated with 2 ml of 4 N NH₄OH, 1 ml of 1.5% 4-aminoantipyrine, and 1 ml of 4% K₃Fe(CN)₆,

quantitatively diluted to 100 ml with H₂O and thoroughly mixed. After 5 min, the reaction was determined colorimetrically at 510 nm and

TABLE 1. Amount of phenol found in the original liquid and rehydrated samples to estimate percentage retained during desiccation

Sample description	% Phenol found in:		% Original phenol retained in desiccated sample
	Liquid sample	Rehydrated sample	
Antiserum	0.52	0.34	65
Antiserum	0.26	0.19	73
Antiserum, concentrate	0.54	0.38	70
Tuberculin	0.45	0.06	13
Pullorum tube antigen	0.61	0.02	3
Albumin solution, 90 mg/ml	0.51	0.41	80
Albumin solution, 90 mg/ml	0.27	0.21	78
Albumin solution, 90 mg/ml	0.11	0.09	78
PBS, pH 5.5, ca. 0.1% phenol ^a	0.10	<0.001	0.4
PBS, pH 5.5, ca. 0.25% phenol	0.24	<0.001	0.2
PBS, pH 5.5, ca. 0.5% phenol	0.54	<0.001	0.1
PBS, pH 7.2, ca. 0.1% phenol	0.10	0.01	7
PBS, pH 7.2, ca. 0.25% phenol	0.25	0.01	4
PBS, pH 7.2, ca. 0.5% phenol	0.53	0.01	3
PBS, pH 8.5, ca. 0.1% phenol	0.10	0.01	6
PBS, pH 8.5, ca. 0.25% phenol	0.25	0.01	4
PBS, pH 8.5, ca. 0.5% phenol	0.55	0.02	3

^a Approximate quantity of phenol added to sample.

compared to standard solutions treated in a similar manner. Samples containing low levels of phenol were retested with modifications of this test, which permitted detection of phenol at levels as low as 0.0005%. Normally, routine samples are assayed using an automated procedure (1), but the manual test is much more versatile when trying to estimate phenol levels over such a broad range of concentration.

The amounts of phenol found in the original liquid and rehydrated samples are presented in Table 1. These values have been averaged and rounded for convenience, but the percentages retained were calculated prior to rounding. The rehydrated biological products retained between 3 and 73% of their original amount of phenol. There was a considerable variation among types of biologics. The highly proteinaceous products, serum and serum concentrates, retained about 70% of their phenol, whereas the products low in protein, tuberculin, retained 13% and the bacterial antigen retained only 3%. Albumin solutions high in protein retained almost 80% of the phenol regardless of the amount originally present. The PBS solutions retained a similar percentage of phenol regard-

less of the amount originally present, although there was a considerable difference due to pH. Evidently the phenol molecule at pH 5.5 is more volatile than the phenolate molecule at pH 7.2 or 8.5, although only a small amount of the phenol was retained by the nonprotein samples.

It is evident that some biological products retain a large percentage of their original phenol during desiccation which could adversely influence certain uses of these products. Caution should be observed when using previously preserved and desiccated biologics in preservative-sensitive test systems. This is especially true of highly proteinaceous biologics.

LITERATURE CITED

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