Diagnostic Antigens and Antisera in Tablet Form

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Diagnostic antigen suspensions and antisera can be prepared in tablet form with no loss in potency.

Berman et al. (1) demonstrated that freeze-dried typhoid vaccine could be prepared in tablet form without loss in potency or stability and suggested that tablets would provide a convenient form for both shipment and storage. Recently, studies were undertaken in this laboratory to determine the feasibility of tableting the bacterial febrile antigens that are prepared here and currently distributed as fluid preparations to armed forces diagnostic laboratories.

The O and H antigens of *Salmonella typhi*, the H antigens of *Salmonella paratyphi* A and *Salmonella schottmuelleri*, and the *Proteus* OX-K somatic antigen were prepared by a modification of the method of Edwards and Ewing (2). Agar-grown organisms were harvested in physiological saline, mixed with an equal volume of absolute alcohol, and held for 18 h at 37°C for inactivation. Another volume of saline was added, and the mixture was thoroughly agitated. The organisms were then collected by centrifugation at $2,000 \times g$ for 1 h and resuspended in 0.85% saline to yield a suspension with a transmittance of 78% ($\pm 0.5\%$) as measured in the Coleman Junior spectrophotometer. The fluid preparations represent concentrated antigen suspensions that are distributed to military laboratories throughout the world, with instructions to prepare a 1:10 dilution in saline for use in the tube agglutination test for the diagnosis of the febrile diseases.

To provide adequate bulk for freeze-drying and tableting, sufficient NaCl was added to the fluid concentrate to yield, on dilution or rehydration for use, the proper concentration of the antigen in physiological saline. This material was distributed in 2-ml volumes in 10-ml serum vials; split butyl stoppers were partially inserted in the vials, and the product was frozen at -60°C in a mechanical freezer. The various antigen preparations were dried from the frozen state over a 48- to 52-h drying cycle, and the vials were sealed under the original vacuum. Individual tablets were made by removing the freeze-dried material from a vial, placing it in a 1-cm die of the tableting machine, and applying

454 kg of pressure to the ram. Each tablet was then placed in a serum vial, a split butyl stopper was inserted, and the vial was sealed under vacuum.

To test antigens, the tube agglutination test, employing homologous rabbit antisera, was used. The fluid concentrates were diluted with 1:10 distilled water containing 0.5% phenol, and the dried powders and tablets were rehydrated with 20 ml of phenolized distilled water. The results of agglutination tests are given in Table 1.

The results indicate that tableting of the freeze-dried antigen preparations can be successfully accomplished on a laboratory scale. Compression or intimate contact of the antigens with the NaCl filler had no adverse effect on agglutinability.

In addition to the above febrile antigens, three freeze-dried antisera, *Salmonella* O polyvalent, *Salmonella* Vi, and *Vibrio comma* Ogawa, available from commercial sources, were also tableted (to produce a solid tablet with these materials, 1,812 kg of pressure on the ram was required). The agglutinating activities of these tableted materials were compared with the dried material from which they were prepared by titrations with homologous living antigens, using the slide agglutination test. The results (Table 2) demonstrate that compression of the freeze-dried powders into tablets did not reduce the agglutinating activi-

TABLE 1. Agglutinability of fluid, freeze-dried, and tableted antigens against homologous antisera

Antigen	Reciprocal of titer		
	Fluid	Freeze-dried	Tablet
<i>Salmonella typhi</i> O	1,280	1,280	640
<i>S. typhi</i> H	1,280	2,560	1,280
<i>S. paratyphi</i> H	640	640	640
<i>S. schottmuelleri</i> H	320	640	640
<i>Proteus</i> OX-K	160	320	320

TABLE 2. Agglutinability of tableted antisera against homologous antigens

Antiserum	Reciprocal of titer	
	Freeze-dried	Tablet
<i>Salmonella</i> O polyvalent	80	80
<i>Salmonella</i> Vi	1,280	1,280
<i>Vibrio comma</i> Ogawa	1,280	2,560

ties of the antisera tested. Furthermore, tablets held for 9 months at 4°C showed no loss in titer, indicating that compression or intimate contact with the NaCl filler had no effect on the stability of these preparations. Additional stability studies are in progress.

These studies indicate that producing diagnostic antigens and antisera in tablet form is feasible and can provide stable, compact diagnostic materials, permitting shipment and storage of multiple units in one container (either bottle-shaped or a flat compact type) and thus conserving space and reducing costs.

LITERATURE CITED

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