AD 672 943

EFFECT OF LYOPHILIZED MEDIA COMPOSITION ON BACTERIAL VIABILITY IN DRY LIVE ANTI-PLAGUE VACCINE

I. V. Pechnikova

Army Biological Laboratories
Frederick, Maryland

17 September 1965

TRANSLATION NO. 1527

DATE: 17 Sept. 1965

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

EFFECT OF LYOPHILIZED MEDIA COMPOSITION ON BACTERIAL VIABILITY IN DRY LIVE ANTI-PLAGUE VACCINE

I. V. Pechnikova (Stavropol')

Mikrobiologiya i Immunologiya Osobo Opasnykh Infektsiy (Microbiology and Immunology of Especially Dangerous Diseases), Saratov, 1964, pp 283-287.

Extensive utilization of live anti-plague vaccine has become possible only after the development of drying methods from a frozen state; i.e., methods of lyophilization (Mudd and Flosdorpf, 1936; Faybich, 1946; Dolinov, 1947). Of great importance for obtaining the dry live anti-plague vaccines are lyophilized media capable of preserving the live bacteria within.

The presently used sucrose-gelatin lyophilized culture medium (1935) is capable of preserving, in a freeze-dried state, up to 50-60% of the live bacteria and to assure preservation of the major part of them for up to two years in storage at 4°C. However, when strict temperature conditions for preserving them are not observed, the amount of live bacteria in the dry live anti-plague vaccine is rapidly reduced. Considering that plague foci are chiefly localized in the southern regions, while the population is usually vaccinated during the warm season, the preservation of a high percentage of live bacteria in the vaccine is decisive.

Under field conditions and in sectors of surgeons and medical assistants it is difficult, as a rule, to create optimum temperature conditions for preserving vaccines; they are often stored at room temperature (18°-22°C), and occasionally at even higher temperatures.

The necessity for creating specific conditions for storing vaccine and the longevity of its existence at an unfavorable temperature have forced us to undertake a search for new lyophilized media with more pronounced protective properties both in the production process and in long-term vaccine storage under refrigeration and at unfavorable temperatures.

Many scientists (Naylor and Smith, 1946; Obayashi, 1960; Muggiton, 1960, and others) have searched for new drying media for lyophilization of various bacteria and viruses, using various chemicals and vitamins, such as dextran, thiourea, sodium glutamate, pentone, ascorbic acid, and many other chemical substances. Our problem is to study the effect of several of these substances on the outcome of live bacteria during the drying of anti-plague vaccine EV from a frozen state, and also on the ability to protect live bacteria from the harmful effects of unfavorable temperatures during storage. In addition, our experiments included ammonium molybdate, which actively affects the redox potential of the medium and also has a stimulatory effect on the growth of plague bacteria in both liquid and solid nutrient media (Gubina, 1962).

The following drying media were prepared and analyzed:

I. Control medium: 1) saccharose 10% + gelatin 1% (according to regulations).

II. Cultures with dextran: 2) saccharose 10% + dextran 3%; 3) saccharose 10% + dextran 3% + ascorbic acid 0.5% + thiourea 0.5% + peptone 0.05%; 4) saccharose 8% + dextran 3% + sodium glutamate 5%; 5) saccharose 8% + sodium glutamate 5% + thiourea 0.5% + peptone 0.05%.

III. Cultures with ascorbic acid: 6) saccharose 10% + gelatin 1% + 0.5% ascorbic acid; 7) saccharose 10% + gelatin 1% + ascorbic acid 0.5% + thiourea 0.5%; 8) saccharose 10% + gelatin 1% + ascorbic acid 0.5% + thiourea 0.5% + peptone 0.05%.

IV. Media containing 5% sodium glutamate: 9) saccharose 8% + gelatin 1% + sodium glutamate 5%; 10) saccharose 8% + gelatin 1% + sodium glutamate 5% + thiourea 0.5%; 11) saccharose 8% + gelatin 1% + sodium glutamate 5% + thiourea 0.5% + peptone 0.05%.

V. Cultures with 1.5% sodium glutamate: 12) saccharose 8% + gelatin 1% + sodium glutamate 1.5%; 13) saccharose 8% + gelatin 1% + sodium glutamate 1.5% + thiourea 0.5%; 14) saccharose 8% + gelatin 1% + sodium glutamate 1.5% + thiourea 0.5% + peptone 0.05%.

VI. Cultures with ammonium modybdate and thiourea: 15) saccharose 10% + gelatin 1% + thiourea 1%; 16) saccharose 10% + gelatin 1% + ammonium molybdate 0.4%; 17) saccharose 10% + gelatin 1% + thiourea 0.5% + ammonium molybdate 0.4%; 18) saccharose 10% + gelatin 1% + ghiourea 1% + ammonium molybdate 0.4%; 19) saccharose 10% + gelatin 1% + thiourea 0.5% + ammonium molybdate 0.4% + riboflavin 0.01%; 20) saccharose 8% + gelatin 1% + thiourea 0.5% + ammonium molybdate 0.4% + sodium glutamate 1.5%.

The lyophilized cultures are prepared sterilly in an isolation chamber, immediately prior to washing the culture, on a saccharose-gelatin medium or in a dextran solution of the same series. Components of the medium are sterilized by brief boiling in 2-3 ml of distilled water and are added to the basic lyophilized culture. The pH of each culture is set at 7.2 by a 10N solution of caustic soda.

A 24-hour test culture of the EV vaccine strain is cultured on meat-peptone agar. After two days at 38°C the culture and separating flask are washed by various lyophilizing media. Density of the bacterial suspension is increased to 70 billion cells per ml (according to the standards of the State Institute for Control of Sera and Vaccines imeni L. A. Tarasevich for turbidity), by using the same lyophilizing medium in which the culture was washed. The suspensions are placed in a refrigerator at +4°C for two days, the time necessary for checking the purity. The suspension is then diluted, 1 ml each in ampules of 6 ml capacity. Conditions of lyophilization were identical in all experiments: freezing at -30°C slowly on a freezing table; time of drying, 16.5-17.5 hours; vacuum, 50-35; heating to 23-30°C; residual humidity, from 2.5-6%. In each experiment all experimental samples were dried simultaneously in one room with a KS-6 apparatus.

Ampules with all of the lyophilized experimental serial vaccines were tested in a vacuum and placed in storage at various temperatures (37°, 28°, 20°, and 4°C). The percentage of bacterial viability in dry vaccine was determined by serial dilution with physiologic salt solution (at room temperature) and with a 0.1 ml culture on three agar plates from a 10^{-7} and 10^{-8} dilutions. The same series of Hottinger's agar with a 0.05% sodium sulfite was used in determining the viability of the bacteria. Cultures were incubated at 28°C for 3-4 days; the number of growing colonies were then counted and the percentage of live bacteria was calculated on the basis that each colony had grown from a single bacterium. Results of the experiments are given in tables 1-3.

As seen from the tables, the lyophilizing media consisting of the dextran solution with the addition of saccharose, ascorbic acid, sodium glutamate, thiourea, and peptone in varying amounts is less in bacterial viability in the vaccine according to quality than the standard saccharose-gelatin lyophilized medium, even when the vaccine has been stored in a refrigerator at 4°C.

The addition of ascorbic acid in various amounts, along with other ingredients, to the saccharose-gelatin medium (0.5%) did not improve its quality, nor did the addition of 5% sodium glutamate, even in combination with thiourea; its quality was only slightly improved with the simultaneous addition of thiourea and peptone.

By adding to the usual saccharose-gelatin medium ammonium molybdate and thiourea or 1.5% of sodium glutamate, thiourea, and peptone, the quality of the medium improved considerably. The minimum percentage required by regulations for live bacteria in using the above-mentioned lyophilized media was maintained in all series of vaccines studied for 8-9 months (observation period) while kept at room temperature, and for 50 days at 28°C and for 16 days at 37°C. With the use of the saccharose-gelatin medium of lyophilization only two of the five vaccine series studied were suitable for use during the four-month period, when preserved at room temperature (20-22°C). At higher storage temperatures (28-37°C) only individual series of vaccine retained 10% of live bacteria for 6-20 days.

TABLE 1

Viability of Bacteria in the Experimental Series of EV Vaccine For Different Periods After Lyophilization and Storage at 37°C

(a) № среды	(b) Число опытов	(c) Станд. млрд. м.т.	(d) Выжило микробов сразу после сушки, %	(e) В последующие дни							
				6	8	10	12	14	16	18	20
1	5	57	37,0	1/7	1/4,7	0/4,1	0/3	0/1,7	0/1,4	—	—
2—5	9	56	23,4	1/4,2	0/2	0/0,4	—	—	—	—	—
6—8	8	64	31,0	1/3	0,2	0/2	0/1,3	—	—	—	—
9—10	3	43	27,8	0/5,8	0/3,2	0/3,6	0/3	—	—	—	—
11	2	52	37,5	2/13,2	2/9	2/10,8	2/10	2/11	1/7,2	—	—
12	2	62	43,5	2/11	2/9	2/10,5	1/6,5	0/4	—	—	—
13	2	63	49,2	2/17	2/22	2/19,5	2/15,5	2/12,1	1/9	1/9	—
14	3	60	52,2	3/19	3/18	3/18,6	3/18,3	3/18,3	3/15	2/12	1/10
15	3	65	45,0	3/16,3	3/17,3	3/16	3/15,3	2/8	—	—	—
16	3	66	42,0	2/10	2/9	2/8,3	2/8,5	—	—	—	—
17	5	64,1	48,0	5/15,8	5/16,4	5/16,4	5/16,4	5/12,6	4/10	3/8,4	—
18	2	63,5	54,5	2/25,5	2/18	2/18	2/17,8	2/17	2/18	1/13	1/9
19	3	65,3	46,0	3/16	3/16	3/16,3	3/15,5	3/13	2/10	2/9	2/7
20	2	59	43,5	2/14,5	2/14,5	2/15	2/15,5	1/13,5	1/9,7	1/8	1/7

Note: For numbers of media, see text; numerator = number of the vaccine series with more than 10% live bacteria; the denominator is the mean percentage of live bacteria; minus -- not investigated.

Key: a--Number of the medium; b--Number of experiments; c--Standard (in billions of bacteria); d--Viable bacteria immediately after lyophilization; e--On subsequent days /after lyophilization/.

TABLE 2

Viability of Bacteria in the Experimental Series of EV Vaccine For Different Periods After Lyophilization and Storage at 28°C

(a) № среды	(b) Число опытов	(c) Станд. млрд. м.т.	(d) Выжило микробов после сушки %	(e) В последующие дни								
				10	15	20	25	30	35	40	45	50
1	5	57	37	4/10,4	4/10	3/9	1/5,5	0/3	—	—	—	—
2—5	9	56	23,4	2/3,4	1/2,2	1/2	—	—	—	—	—	—
6—8	8	64	31	—	—	1/1,6	—	—	—	—	—	—
9—10	3	43	27,8	2/14	2/13,6	1/12	0,5	0/4	—	—	—	—
11	2	52	37,5	2/18	2/14,5	2/14,5	1/10	1/10,5	1/5,5	1/5,5	—	—
12	2	62	43,5	—	—	2/18	2/13,2	1/12,8	1/11,2	—	—	—
13	2	63	49,2	—	—	2/25	2/21	2/21,2	2/20,5	2/18	2/17,5	—
14	3	60	52,2	—	—	3/30	3/26,6	3/25	3 23,6	3/24,6	3/22	3/15
15	3	65	45	—	—	3/23,3	3/22	3/18,3	3/16,6	2/12	—	—
16	3	66	42	—	—	3/15	2/13,3	1/10	1/9	1/9	1/6,3	1/6,5
17	5	64	48	—	—	5/22,2	5/15,4	5/16	5/16,7	5/14,2	3/16	3/14
18	2	63,5	54,5	—	—	2/30	2/28	2/25,5	2/26,5	2/30	2/25	2/24
19	3	65,3	46	—	—	3/24	3/20	3/14,3	3/16,3	3/18	3/15	2/10
20	2	59	43,8	—	—	2/26	2/27	2/23,5	2/20,5	2/19	2/14,2	1/10

Note: Same as in Table 1; Key: Same as in Table 1.

TABLE 3

Viability of Bacteria in the Experimental Series of EV Vaccine For Different Periods After Lyophilization and Storage at 18°-22°C

(a) № среды	(b) Число опытов	(c) Стандарт. вар. в т.	(d) Выжило микробов сразу после сушки, %	(e) В последующие месяцы							
				3	4	5	6	7	8	9	10
1	5	57	37,0	2/8,4	2/5,4	1/4	—	—	—	—	—
2–5	9	56	23,4	1/6	0/4	0/2,2	—	—	—	—	—
6–8	8	64	31,0	2/5	1/2	—	—	—	—	—	—
9–10	3	43	27,8	2/13,7	2/13	2/12	0,4,6	—	—	—	—
11	2	52	37,5	2/20,6	2/20	2/19	1/8,5	1/8,5	1/9	1/7	0/3,5
12	2	62	43,5	2/14,5	0/7	0/5,2	0/3,5	—	—	—	—
13	2	63	49,2	2/22,9	2/14	2/14	2/15,7	1/11	1/8	—	—
14	3	60	52,2	3/25	3/19	3/19	3/16	3/12	3/14,3	1/10	—
15	3	65	45,0	3/18	3/16,6	3/14	2/14	2/13	2/9,5	—	—
16	3	66	42,0	2/15	1/10,5	0/8	0/7	—	—	—	—
17	5	64	48,0	5/28,4	5/21	5/21,6	5/15	5/15	5/16	5/13	—
18	2	63,5	54,5	2/29,2	2/29	2/30,5	2/30	2/25	2/28	2/24	—
19	3	65,3	46,0	3/23	3/14	3/15	3/14	3/14	2/13	—	—
20	2	59	43,8	2/28	2/23,5	2/23	2/20,5	1/13,2	—	—	—

Note: Same as in Table 1; Key: Same as in Table 1.

Thus, disturbance of the temperature conditions in transporting and storing vaccines is less indicative of the amount of live bacteria in dry live anti-plague vaccine by comparison with our other tested media for lyophilization; these vaccines were prepared on cultures with molybdenum and sodium glutamate, thiourea, and peptone. After nine months of storage at 4°C no marked reduction was observed in the viability of bacteria in the dry live anti-plague vaccine prepared on all media of lyophilization which were investigated.

Conclusions

1. Lyophilization media containing 0.4% ammonium molybdate, 1% thiourea; or 1.5% sodium glutamate, 0.5% thiourea, and 0.05% peptone contributed to longer preservation of live bacteria in a dry anti-plague vaccine with an unfavorable (high) storage temperature.

2. With a storage temperature of 37°C the vaccine prepared on these lyophilization media retained the minimum percentage of live bacteria for 16 days; at 28°C — for 50 days, and at room temperature, for 8-9 months.

Bibliography

Gubina, N. Ye., Zhurnal Mikrobiologii Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology), 1962, 10;

Dolinov, K. Ye., Byulleten' po Obmenu Opytom Institutov Epidemiologii i Mikrobiologii SSSR (Bulletin on Exchange of Experience of Institutes of Epidemiology and Microbiology of the USSR), 1947, 4/22;

Faybich, M. M., Zhurnal Mikrobiologii Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology), 1946, 6;

Muggleton, P. W., Recent Research in Freezing and Drying, edited by Parkers and Smith, 1960;

Mudd, S., and Flosdorf, E. W., J.A.M.A., Sept. 19, 1936, v. 107.

Naylor, H. B., and Smith, P. A., J.Bact., 1946, 52, 5;

Obayashi, V., Recent Research in Freezing and Drying, edited by Parkers and Smith, 1960.