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The 

DEPARTMENT OF THE ARMY Fort Detrick Frederick, Maryland

THE USE OF CULTURING AND COAGULATION FOR THE DETECTION OF PATHOGENIC MICROBES IN WATER

Following is a translation of an article by Junior Scientific Worker Ye.N. Hilyayeva and Candidate of Medical Sciences G.M. Fisher from the Kuybyshev Scientific Research Institute of Epidemiology, Microbiology, and Hygiene in the Russian-language periodical <u>Gigiyena</u> <u>i Sanitariya</u> (Hygiene and Sanitation), No 10, Moscow, 1962, pages 57-58. The article was submitted for publication on 27 September 1961.

The existing methods of detecting pathogenic microbes in water are rather complex, laborious, and require special apparatus (centrifuge, vacuum pumps, bacterial filters, etc.) or coagulating substances (green vitriol, ferric chloride, alum, etc.).

In our work we employ a simpler method which was developed by one of the authors (G.M. Fisher) which consists of the following. 450 milliliters of water are poured into a sterile dish. Chlorinated water is dechlorinated. 50 milliliters of 10% peptone (pH = 8.0-8.2) is added to the sample. Thus the concentration of peptone in the test water becomes 1%. The sample is then placed in a thermostat at a temperature of 37

degrees Centigrade. After three hours 2.5 milliliters of a 10% solution of potash alum is added to the water. During the time the water is in the thermostat coagulation occurs and a flocculent precipitate is formed on the bottom of the dish. It should be noted that after 15 to 20 minutes large flakes of the coagulant rise to the surface and therefore the mixture must be agitated. . After an hour the dish is removed from the thermostat and the clear part of the liquid is poured off so that 100 to 200 milliliters of the liquid with the precipitute remains. The remaining liquid is filtered through a sterile piece of filter paper. The precipitate which is obtained in the form of a pasty mass is removed from , the filter paper with a glass rod and is transferred to five Petri dishes with Ploskirev's culture medium and is pulverized with a spatuala. During the summertike when the water terperature is high (18-20 degrees Centigrade), the culturing should be shortened to one hour. It was established experimentally that the detection of pathogenic microbes using the indicated method is successful for concentrations of 100 microbe bodies per liter of water.

The indicated method was used to investigate 199 samples from different water sources. In 9 samples (4.52%), the pathomens of dysentery and paratyphoid fever were isolated in a pure culture (see the Table).

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Place where sample was obtained	No of samples tested	(oli-titer	No of positive sampler	Types of pathogenic microbes isolated
River tap water, not purified /See note/	66	0.01-11.11	2	Flexner's and Newcastle's dysentery bacilli
Purified tap water /See not	18 e/	22.2-111.1	-	-
Water from the Volga	45	0.01-11.11	2	Flexner's and Newcastle's dysentery bacilli
Water from the Samara	9	0.0005- 0.006	1	Typhoid bact. ria
Water from the Sok	33	0.46-111.1	-	-
Water from tuoular wells	23	3.6-250	3	Paratyphoid 1 bacteria,
			· · ·	Flexner's and Newcastle's dysentery ba
Sewer water	5	-	1	cilli Typhoid bacteria
Total.	199	-	9°,	-

The cultures which were obtained had the following cultural properties. Three strains of Flexner's dysentery were biochemically typical in their action and rave a positive reaction to acqlutination with Flexner's dysentery serum but were phatoresistant. One of the strains mave a positive result with Flexner's A-type serum. Three Newcastle strains had all the typical biochemical and serological properties. Two strains of typhoid were typical and mave a reaction to anglutination with Vi and O serums and were able to undergo phagolysis. The isolated strain of paratyphoid B, bosides the typical biochemical properties, gave a positive reaction with monoreceptor serums O antigen (I, IV, V) and N antigen (b,1,2).

In addition, 8 atypical cultures were isolated, the study of which in 6 months indicated that these cultures were para-intestinal bacilli.

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