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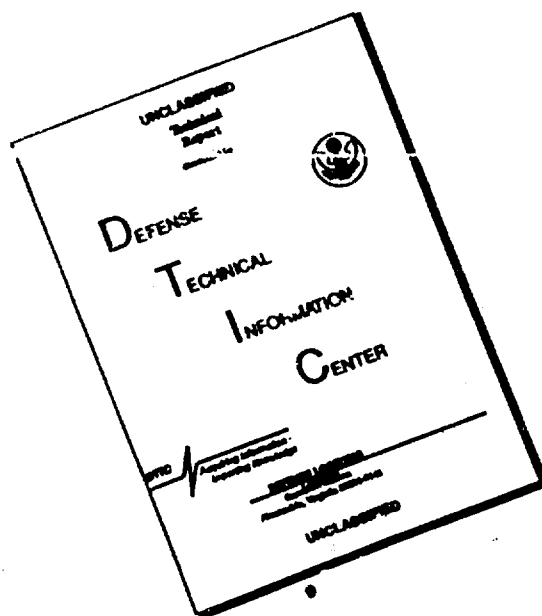
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Trudy Tomskovo Nauchno-Issledovateleskogo Instituta Vaktsin i Syvorotok, Ministerstva Zdravookhraneniya, USSR (Transactions of the Tomsk Scientific Research Institute of Vaccines and Serums, Ministry of Health, USSR), vol.12, 1960, pp.140-144

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Quick Diagnosis and Sanitary Expertise of Water Contaminated by Pathogenic Microbes or their Toxins

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A sanitary expert examination of water presents a time consuming and important task performed by health officers together with epidemicologists and bacteriologists.

There are very many methods of detection and identification of microbes or their toxins. In examination of water one must use the methods which offer the most complete and reliable data in the shortest possible time.

Much consideration has been and is still given here and abroad to the problems of a quick diagnosis and identification of pathogenic bacteria in natural medium.

The most promising are the methods of quick diagnosis that are based on the use of polysaccharide-hapten in seroreactions. This substance, as we know, is the only carrier of serological specificity of most pathogenic bacteria.

We, in the Soviet Union, have developed a method of this reaction with 1 (O)-group of human erythrocytes, as reported by

KRAVCHENKO and SOKOLOV (1). In addition to the positive sides, this technique also has a number of important disadvantages. Its basic negative factor is that, being a living biological substrate, human erythrocytes die away rather quick and are destroyed outside the organism; even a slight hemolysis accompanying this process causes a spontaneous agglutination, which is indistinguishable from the agglutination yielded by specific serums. Sometimes, the same autoagglutination begins because erythrocytes possess their antigenic nature. One can prevent, for some time, a quick destruction of erythrocytes by keeping them in the cold.

The use of banked blood and desiccated stromal erythrocytes reduces the sensitivity of reaction.

A.V. ORLOV (4) proposed to use cholesterol that possesses no natural antigen, but is a component of stromal erythrocytes; it has long since been used in the serological diagnosis of syphilis as an ingredient intensifying the sensitivity of antigens.

The author named his reaction "haptocholic flocculation in vitro". Its essence is the same as that of the reaction of KRAVCHENKO and SOKOLOV. The distinctive feature is the substitution of alive, very unstable and costly adsorbent [1 (O)-group of human erythrocytes] by a chemical ingredient (cholesterol) which can be kept in a powder form for years.

We checked the sensitivity and specificity of the reaction of KRAVCHENKO and SOKOLOV using 1 (O)-group of human erythrocytes; we also checked the haptocholic flocculation reaction in vitro proposed by A.V. ORLOV.

To prepare polysaccharide-hapten for use in our experiments,

Table 1

Findings on Sensitivity of flocculation reaction in vitro compared with those of the KRAVCHENKO-SOKOLOV Reaction with Pure Haptens of Dysentery Group and with those of the Mixture of Added Coliform Bacteria

		Initial concentration of microorganisms in 1 ml of investigated fluid (in millions)									
		Haptocoholic flocculation in vitro					KRAVCHENKO-SOKOLOV reaction				
		25	50	100	200	25	50	100	200	250	
FLEXNER'S dysentery bacteria		--	+	++	++++		++	+++		200	
SCHMITZ-SCHUTZER'S dysentery bacteria		+	++	+++	++++	+	+++	++++		200	
SONNE'S dysentery bacteria		+	+	+	+++	+	++	+++		200	
Mixture of polysaccharide-haptens of dysentery and coliform bacteria added proportionally		1:5	+	++	++++	+	++	+++		200	
		1:10	+	++	++++	+	++	+++		200	
		1:15	-	+	++	++++	-	++	+++		200
SCHMITZ-SCHUTZER'S dys. bacteria		1:5	++	+++	++++	++	+++	++++		200	
		1:10	+	++	+++	++++	+	+++	++++		200
		1:15	-	++	+++	++++	-	+++	++++		200
SONNE'S dysentery bacteria		1:5	+	++	++++	+	++	+++		200	
		1:10	+	++	+++	++++	+	+++	++++		200
		1:15	-	+	++	+++	-	++	+++		200

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we obtained FLEXNER'S dysentery bacteria "C", No.39 then SCHMITZ-SCHUTZNER'S and SONNE'S dysentery bacteria, also coliform bacteria (KOCHEVNIKOV) No.1 and 5, as well as coliform bacteria .290.

Polysaccharide-hapten was prepared according to the modified SOKOLOV method from bacterial suspensions in water contained from 25, 50, 100 and 200 million to 1 billion of organisms in 1 ml.

The essence of modification in the preparation of polysaccharide-hapten according to SOKOLOV is confined to our use of a smaller quantity of alcohol and, thereby, higher concentrations, this expressed itself more fully in osmotic aspects of solutions. The method of preparation has been described in detail by SOKOLOV (6) and A.V. ORLOV.

The densification of suspension was determined according to the optimum intestinal standard.

Polysaccharide hapten was prepared up in mixture in quantities of 5, 10 to 15 times greater than those sought for.

We also developed in all experiments KRAVCHENKO-SOKOLOV'S reaction concomitantly to ORLOV'S method of the leptocholic flocculation reaction in vitro.

The volume of liquid in which the adsorption process took place was 5 ml. Partial reactions were also accomplished in 12.5 ml volume for comparison purposes.

Serum diluted 1:10 was used with the titration of FLEXNER'S polyvalent - 12,800; SCHMITZ-SCHUTZNER'S - 25,600 and SONNE'S - 6,400.

In all, we performed 48 experiments with pure polysaccharide haptens of dysentery and 108 experiments with a mixture of added

coliform bacteria. Collective experimental results are presented in Table 1.

As we see from Table 1, the haptocholic-flocculation reaction in vitro was positive in the presence of a 50 million concentration of microorganisms in 1 ml of initial suspension in the case of SCHMITZ-SCHTUTZER'S dysentery bacteria; also in the presence of 100 millions of FLEXNER'S dysentery bacteria and 200 millions of SONNE'S dysentery bacteria. The experiments with the KRAVCHENKO-SOEGLOV reaction were affirmative in the presence of 25 million concentration of microorganisms in initial suspension of SCHMITZ-SCHTUTZER'S dysentery bacteria and with 50 million concentration of FLEXNER'S and SONNE'S dysentery bacteria.

Dissimilar data pertinent to the sensitivity of both reactions with various types of dysentery bacteria are, apparently, influenced by various factors of used serums.

The evaluation of the haptocholic flocculation in vitro was hindered by insufficient homogenization of cholesterol in the physiological solution after centrifugation and in serums during the performance of experiments. In connection with the above, we considered a positive reaction with such concentrations of haptens, which would make quite impossible to confuse a specific and non-specific conglomeration of haptocholic suspension in vitro during basic experiments and also under control conditions. In the reaction evaluated by us as positive, the agglutination of suspended cholesterol particles was clearly visible, also their enlargement and a distinct clarifying of fluid were apparent. We rated such reaction evaluation by two crosses (++); three crosses (+++) were

indicated in cases in which the agglutination, the enlargement of cholesterol particles and the clarity of fluid were still better expressed. In the presence of massive clottings and complete clarity of fluid, the reaction was rated by four crosses (++++). All reactions with cholesterol were examined behind a black background within 2 to 5 minutes. Reactions after 5 minutes were disregarded.

The KRAVCHENKO-SOKOLOV reaction is more pliant in a sense of evaluation, consequently it offers an opportunity for detection of erythrocytes' agglutination at much lower concentrations of haptens, i.e. when fresh human erythrocytes are used of the 1 (C)-group and have been washed just 2 or 3 times. Erythrocytes kept in the cold for 4 or 5 days, or at room temperature for one day may give a spontaneous agglutination, which could be prevented by washing erythrocytes at least 4 times.

Thus, we accepted as positive the KRAVCHENKO-SOKOLOV reaction and rated it by two crosses (++) , while applying three crosses (+++) to the haptocholic flocculation in vitro.

The addition of heterogeneous antigens to tested substances in proportions of 1:5, 1:10 and 1:15 failed to have a noticeable effect on the sensitivity of both reactions.

The specificity of the haptocholic flocculation reaction in vitro and that of the KRAVCHENKO-SOKOLOV reaction has been confirmed in numerous experiments with the reciprocal agglutination involving heterogeneous and homologous serums.

The number of positive reactions increased in solutions containing 25 to 50 millions of microorganisms, following the in-

crease from 5 to 12.5 ml of the volume of fluid in which the adsorption process took place, while the concentration of microorganisms remained unchanged in 1 ml. The same effect was not observed in mixtures containing much higher concentrations of microorganisms.

The contact photographing helped us in reaction readings and in computation of the reaction intensity during our research; it could also be successfully used in a practical performance similar to the procedure with the diaphragm filters at the waterworks laboratories to determine the indexing of water.

Our verification of the improved reaction method suggested by A.V. ORLOV and involving haptocholic flocculation with the use of diaphragm ultrafilters, fully confirmed the findings of the author, in that cholesterol, as a suspended matter, is more stable in suspensions. There is an obvious and greater reaction sensitivity reflected by way of a fuller adsorption of polysaccharide haptens from mixtures.

#### Conclusions

1. Seroreactions with polysaccharide-haptens are sufficiently sensitive and can be fully used in diagnoses of pathogenic bacteria during expert examination of water.

2. In practice, the haptocholic flocculation reaction in vitro is not inferior to the KRAVCHENKO-SOKOLOV reaction.

3. It is essential to examine much closer the mechanism of the haptocholic flocculation reaction in vitro and to search for such colloidal system, which would give more homogeneous and stable suspensions of cholesterol.

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Summary Printed in English

Reaction of haptocohic flocculation on the glass with polysaccharide hapten of Flexner's dysentery bacteria "C", No.390, Sturzer-Shmitz and Sonne, put forward by A.B. Orlov, does not practically yield in its sensitivity to Kravchenko-Sokolov's reaction.

The flocculation of cholesterol and erythrocytes charged with polysaccharide hapten set in, as a rule, only under influence of specific sera.