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PERTUSSIS VACCINE IN THE AIR

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A METHOD FOR DETERMINING THE CONCENTRATION OF KILLED PERTUSSIS VACCINE IN THE AIR

[Following is a translation of an article by F. G. Mayorova, Gamaleya Institute of Epidemiology and Microbiology, AMN, USSR, published in the Russian-language periodical Zhurnal Mikrobiologii Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology, and Immunobiology) 1963, No 11, pages 65-69. It was received on 2 Mar 63. Translation performed by Sp/6 Charles T. Ostertag Jr.]

One of the necessary conditions for utilizing the inhalation method of immunization is the presence of a method for determining the concentration of vaccine in the air and the inhaled dose. Since under these conditions of work it is necessary to deal with low concentrations of antigen, the method for determining it should be highly sensitive.

The mission of the present work is developing a method for determining killed pertussis vaccine in the air. With this aim an attempt has been made to use the complement fixation reaction in the classic arrangement.

Immune rabbit antipertussis serum produced by the Moscow Institute of Microbiology, Epidemiology, and Hygiene was used for the reaction. Before the experiment the serum was warmed for 30 minutes at 56° (inactivation of complement) and its working dilutions prepared in a physiological solution (1:50 or 1:70). The complement was titrated before the experiment and taken in the amount of 1.5 and 2 units (as a unit of complement we accepted that amount of it which was necessary for the lysis of an 0.01% suspension of erythrocytes in 0.25 ml mixed with 0.25 ml of a threefold titer of hemolysin). The vaccine from pertussis bacteria phase I which are formalin killed served as the antigen.

The reaction was taken into consideration after it was carried out in control test tubes and was considered as positive with a clear impediment of hemolysis (+++ and ++++).

The complement fixation reaction turned out to be suitable for the quantitative determination of pertussis vaccine in low concentrations. As an example, we will present the results of one experiment (table 1) which

showed that in this it was possible to expose 250-312, 250-500 million microbial cells in 1 ml of suspension. The specified fluctuations were apparently caused by the lability of the biological components of the reaction, and maybe by a different calibration of the pipettes. In this, it turned out that in the reaction it was more advantageous to use not 2 but 1.5 units of complement, in the presence of which the reaction was more sensitive.

In determining the concentration of pertussis bacilli in each experiment the suspension of vaccine being tested was titrated parallel with a suspension of vaccine in which the concentration of pertussis bacilli was established earlier according to the optical standard (control series).

The content of pertussis antigen was computed according to the extreme dilutions which inhibit hemolysis (+++ and ++++), considering that the concentration of extreme dilutions of the control and test series is equivalent. We will present (table 2) examples of the calculation based on the data from a number of experiments. The computation was carried out in the following manner. It is known that in the control, the pertussis vaccine in the initial concentration, equal to 2 billion microbial cells in 1 ml, was established to a dilution of 1:4, that is to 500 million microbial cells in 1 ml. The test solution reacted down to a dilution of 1:8, or 1:16 or 1:32, etc., consequently 1 ml of test solution contained 500 million $\times 8 = 4$ billion, or 500 million $\times 32 = 16$ billion, etc. The results obtained cannot be indicators of the absolute concentration of vaccine, since their determination was limited by the indicators of the series of dilutions (1:2, 1:4, 1:8, 1:16, etc.). By reducing the gap between dilutions the difference in the concentrations being exposed can be lowered.

With this aim of consolidating and refining the determination of the concentration of pertussis vaccine, we utilized a FEK-M photocolorimeter. For this a graduated curve was constructed which corresponded to the various concentrations of formalized pertussis (fig. 1). In constructing the graduated curve, suspensions of vaccine were used with a content of from 50 million up to 3 billion of microbial cells in 1 ml based on the pertussis standard. Measuring the optical density of all the suspensions was done with a blue light filter while using a cuvette 2 mm in diameter. Determining the optical density of a solution of pertussis bacilli of an unknown concentration was performed on the blue light filter. Then, based on the graduated curve the concentration of suspension was found which corresponded to the given optical density.

Determining the optical density of the vaccine parallel with setting up the complement fixation reaction verified the correctness of the results. The exposure of the concentration of vaccine based on optical density turned out to be very accurate and reflected small changes in concentrations. In practice, by this method it was possible to determine a concentration of pertussis vaccine approximately down to 50-100 million bacteria in 1 ml (based on the pertussis standard).

Both methods were used for determining the concentration of an aerosol of pertussis vaccine in the air of an aerosol chamber. The pertussis bacilli were trapped on FPA fibrous filters with a resistance of 1.5--2 mm water column. The microbial cells were so densely fixed on the fibers that for the complete exposure of all the antigen found on the filter it was dissolved in acetone. The pertussis vaccine was separated out during centrifugation. The centrifugate was maintained in the test tubes for no less than two hours (some times up to the following day) at room temperature until the evaporation of the acetone residue and then suspended in a physiological solution. With this suspension the complement fixation reaction was set up and the concentration of microbial cells was determined by photolorimetry. It was established that when mixing the acetone with the physiological solution turbidity appeared, therefore it was necessary to carefully watch that after centrifugation the acetone completely evaporated. These manipulations didn't reflect substantially on the pertussis antigen (fig. 2 and 3). After staining the preparations with antiluminescent pertussis serum in a dilution of 1:40 the brightness of luminescence was preserved (++++).

The calculation of the concentration of vaccine (K) in 1 ml of air was carried out by computing the liters of air passing through the filter (V), the dilution in the test series in which the pertussis antigen was determined (C), and the final concentration of the control series (A) according to the formula:

$$K = \frac{A \cdot C}{V}$$

Calculation of the inhaled dose (D) was carried out by computing the instantaneous lung volume of the subject (m), the concentration of vaccine in 1 liter of air (K), and the coefficient of aerosol inhibition with a value up to 3μ in the respiratory tracts (g) according to the formula.

$$D = K \cdot m \cdot g$$

CONCLUSIONS

1. The method of the quantitative determination of pertussis bacilli in a suspension with the help of the complement fixation reaction is sufficiently sensitive and reliable. With this method it was possible to expose down to 250-500 million pertussis bacilli in 1 ml.
2. For determining the concentration of pertussis bacteria in an experiment it is recommended to use the photolorimetric method under the control of reactions which expose its specificity.
3. The complement fixation reaction and the photolorimetric method can be used for determining the concentration of pertussis bacilli in the air of a room and the extent of the inhaled dose of pertussis vaccine.

Figure 1. (page 67) Photocolorimeter indicators depending on the concentration of the microbial suspension.

- a. Photocolorimeter indicators
- b. Pertussis bacilli
- c. Intestinal bacilli
- d. Concentration of microbes (in billions)

Figure 2. (page 68) Preparation of pertussis vaccine retained from the air by a FPA filter. Stained with specific luminescent pertussis serum.

Figure 3. (page 68) Preparation of pertussis vaccine from the precipitate obtained after dissolving of the FPA filter with acetone. Stained with specific luminescent pertussis serum.