

Conditions for Negative Staining of Influenza Virus on Carbon Coated Microgrids

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ABSTRACT : The negative staining for the electronmicroscopic preparation of virus particles was made by adjusting the surface tension of carbon-coated grids with potassium hydroxide. The fine structure with good contrast appeared in the smooth background of the film, when the specimen was stained with the 2% phosphotungstic acid solution containing 1/30 N potassium hydroxide.

There are several important requirements for the negative staining of the virus particles to produce the good results. One of the conditions is to spread the virus particles on the grids. It may be depend upon the surface tension of the grids, particularly carbon-coated grids. In this short communication, the conditions for the single negative staining of influenza virus with phosphotungstic acid solution are presented.

MATERIALS AND METHODS

Virus and instruments: Influenza virus in alantoic fluid was purified and concentrated by the sedimentation with Sharpres centrifuge. A paraffin plate with a number of dents in small size (0.5 mm in diameter) was placed on ice pieces kept in a vessel (20 cm of length and width). The paraffin plate was covered with a plastic box (Fig. 1). The moist chamber for the fixation and the negative staining of the specimens on the grids was shown in Fig. 2.

Reagents: 0.1 M sodium cacodylate-HCl buffer containing 1% CaCl_2 and 0.75% saccharide was prepared for washing the specimens on the grids and also for the solvent. Four percent paraformaldehyde solution in 0.1 M cacodylate buffer containing 1% CaCl_2 and 0.75% saccharide was used for the fixation of the virus particles on the grids. For the negative staining, five series solution of phosphotungstic acid (PTA) were prepared as follows : 3 ml of 2% PTA solution was added with (1) 0.05 ml of 1 N KOH, (2) 0.1 ml of 1 N KOH, (3) 0.15 ml of 1 N KOH, (4) 0.2 ml of 1 N KOH, (5) 0.5 ml of 1 N KOH

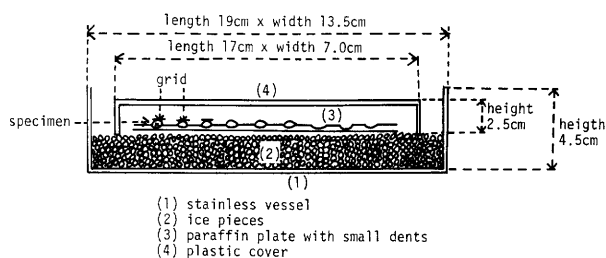


Fig. 1 Ice Vessel

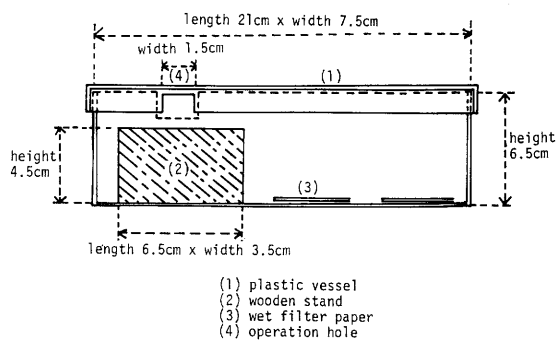


Fig. 2 Moist Chamber

respectively.

Procedure: The virus suspension was dropped in the dent of paraffin plate and kept gently for a few minutes. The film face of grid was put on the drop of virus suspension and kept for a few minutes. After the residual virus solution was blotted with a piece of filter paper, the film face of grid was put on the drop of cooled cacodylate buffer in the dent of paraffin plate and kept for a few minutes by slightly moving the grid. This procedure for washing the specimen on the grid was repeated twice. The fixation of the virus particles on the grid was made by the similar procedure described above. The film face of a grid adhered the virus particles was put on the drop of cooled 4 % paraformaldehyde solution in cacodylate buffer and kept for 10 minutes. After fixation, the grid was washed on the drop of cooled water for a few minutes by slightly moving the grid. The washing procedure was repeated 5 times by changing the drop of water. The sufficiently washing the grids was necessary for the avoid of crystallization. The film face of grid was put on the drop of PTA solution for a few minutes. After the residual PTA solution was blotted with a piece of filter paper, the grid was replaced immediately on the wooden stand in the moist chamber and kept for 10 minutes. Then, the specimen was observed electronmicroscopically.

RESULTS

The images of the virus particles could not appear clearly in order to hiding the spread of PTA on the film (Photos. 1, 2, 5 and 6).

The good results were obtained in case of staining with 2 % PTA containing 1/30 M or 1.5/30 M KOH (Photos. 3 and 4).

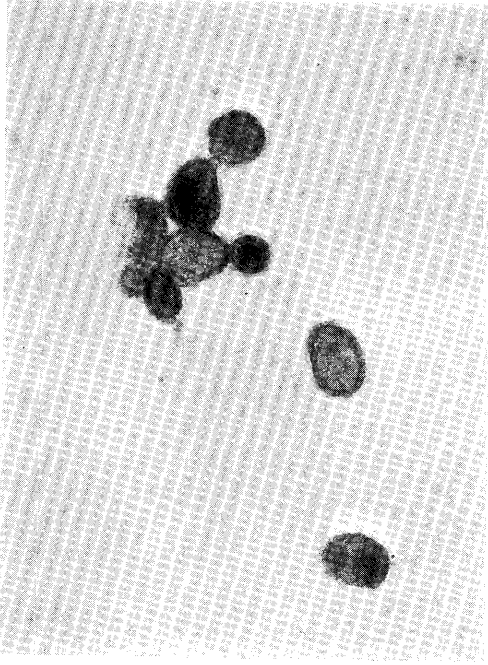


Photo. 1

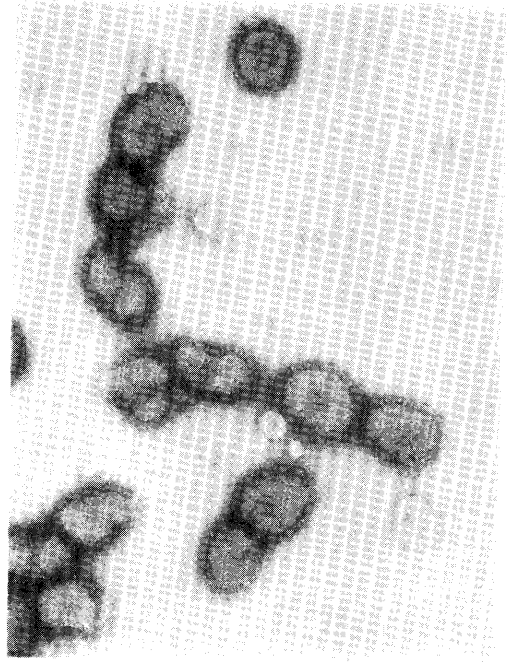


Photo. 2

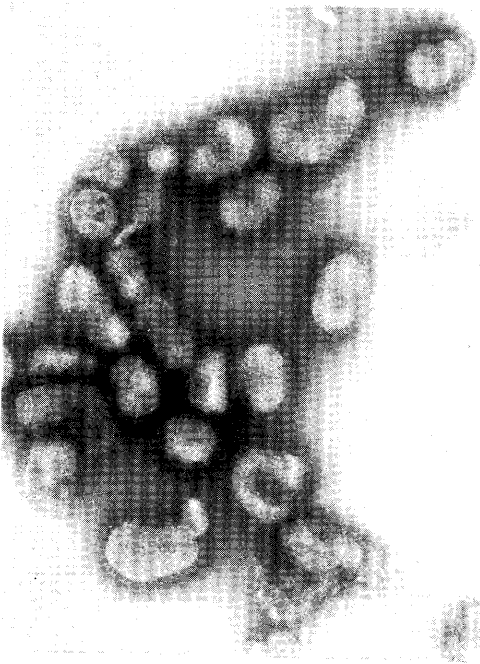


Photo. 3

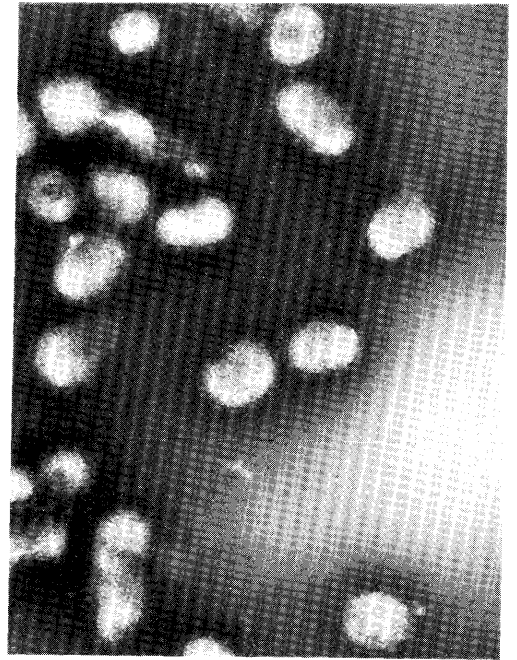


Photo. 4

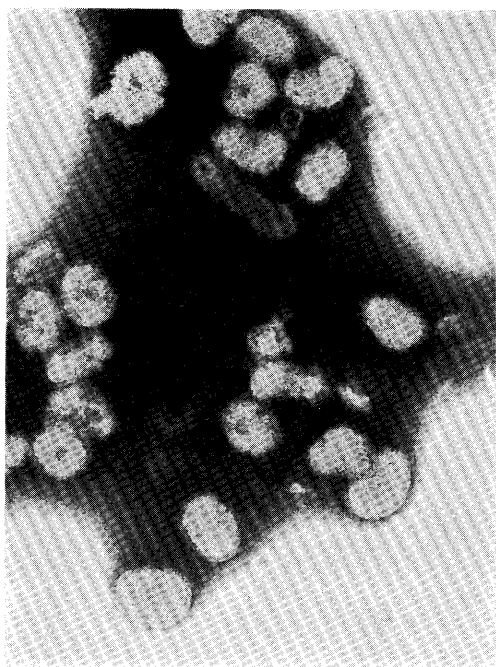


Photo. 5



Photo. 6

Explanation of photographs

- Photo. 1. The image of influenza virus stained with 2 % PTA without KOH
- Photo. 2. The image of the virus stained with 2 % PTA containing 1/60 M KOH
- Photo. 3. The image of the virus stained with 2 % PTA containing 1/30 M KOH
- Photo. 4. The image of the virus stained with 2 % PTA containing 1/20 M KOH
- Photo. 5. The image of the virus stained with 2 % PTA containing 1/15 M KOH
- Photo. 6. The image of the virus stained with 2 % PTA containing 1/5 M KOH

COMMENTS

The advantage procedure for the negative staining was made by many workers. The first requirements for the negative staining were to spread the virus particles and the stain reagents on the film. The attempt to answer this requirement was made usually by adding a small volume of glucose, saccharide or glycerol to the stain reagent solutions (Valentine *et al.*, 1965 ; Horzinek *et al.*, 1969, 1971 ; Nermut, 1972). The spread and contrast of the specimens on the film might also be influenced by the intensity of the ionic dissociation of a certain stain reagent. In this experiment, it was noted that the good conditions for the negative staining with 2% PTA containing 1/20 M or 1/30 M KOH and 0.75% saccharide were selected. The procedure in moist chamber introduced in this experiment could be recommended to obtain the constant results for the negative staining.

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カーボン膜マイクログリッドによるウイルスのネガティブ染色の条件の検討
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ウイルス粒子のネガティブ染色法は多くの先人によってすぐれた方法が紹介されている。しかし、いずれの方法でも膜面におけるウイルス粒子の展開と染色液の伸びになお難点がある。著者等は最も普通に用いられるカーボン膜マイクログリッドを用い、染色液に加える水酸化カリの量を変えることによって染色液の膜面上での伸びが著しく変化することを知り、その条件を検討した。2% 燐タングステン酸ソーダ (PTA) 液に 1/30N になるように水酸化カリを加えた場合、膜面上の PTA の伸びは最もよく、従ってウイルス粒子の微細構造も明瞭であった。

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