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PTA preservation of the serological activity in virus suspensions – also useful for consignments of samples over long distances

Bevaring af den serologiske aktivitet i viruspartikler, opbevaret i fosfor wolframsyre – en metode også anvendelig ved forsendelser af virusprøver over lange afstande

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Summary

Serological activity is kept unchanged towards the homolog virus in virus suspensions in Phosphorous Tungstic Acid (PTA) and Ammonium Molybdate (AM) both 2% (pH 6.8), stored in a refrigerator for up to 10 years.

This opens up possibilities of keeping a »Bank« of virus suspensions as a control of antisera and antisera dilutions used in the ISEM work (immuno sorbent electron microscopy).

It is discussed if this preserving method could also be used by developing countries, with moderately equipped laboratories, as a solution for keeping samples intact under shipment over long distances to electron microscope laboratories (EMLAB).

Key words: PTA-preservation, ISEM.

Resumé

Viruspartikler opbevaret i elektronmikroskopiske farvereagenser som fosfor wolframsyre (PTA) og ammonium molybdat (AM) begge i 2% ved pH 6.8, har vist sig at bevare den serologiske aktivitet i op til 10 år.

Dette åbner mulighed for at have en permanent »virusbank« til kontrol af de mange antisera og antisera fortyndinger, som anvendes i elektronmikroskopisk serologisk arbejde, den såkaldte immuno sorbent elektronmikroskopi (ISEM).

Det diskuteres, om denne opbevaringsmetode også kan anvendes af udviklingslandene som en forsendelsesmetode af prøver til elektronmikroskopiske laboratorier (EMLAB) over lange afstande og vanskelige forhold. Foreløbige forsøg af denne art fra Mocambique og Zanzibar til Danmark har været tilfredsstillende.

Nøgleord: ISEM, PTA-opbevaring.

Introduction

Since the introduction of immuno sorbent electron microscopy (ISEM) it has been of great interest to keep control of the antisera and antisera dilutions used in ISEM work.

It was discovered, that virus suspensions stored in PTA or AM (2%) maintained the serological activity for years (up to 10 years).

Based upon results of 9 different virus suspensions stored this way, it has been discussed if it would be possible always to have virus suspensions in stock, which could be used as a control, both of the antisera and antisera dilutions used in the laboratory. Dilutions of antisera are often unused but disposed of. With a permanent »virusbank« it should be possible to keep control of these antisera dilutions for further use and in this way save expensive antisera stock.

It is of great interest, if this method of keeping the serological activity makes the method useful for consignments of virus samples prepared in the field on their way to well equipped laboratories (EMLAB) over long distances. This could be of interest for developing countries with poorly equipped laboratories. Experiments with this method of consignment will proceed in the coming years. Samples from Mocambique and from Zanzibar have shown promising results already.

Material and methods

9 virus suspensions from virus infected leaves and from purifications of virus were kept in 2% PTA and in 2% AM both at pH 6.8 over a period of years (see table 1) in a refrigerator running at 4°C. The samples were stored in micro test tubes sealed with a rubber stopper.

The old virus samples were tested in 1982 by the ISEM method. The results are listed in table 1 and illustrated in Figs. 1–8.

Preparation for ISEM

All samples were examined with ISEM trapping (*Derrick,* 1972; *Derrick,* 1973; *Bralansky & Derrick,* 1974; *Roberts & Harrison,* 1979; and *Lesemann et al.,* 1980) and ISEM decoration (*Milne & Luisoni,* 1977). The antisera were kindly supplied by *B. D. Harrison* (Dundee), by *H. L. Paul* (Braunschweig) and by *M. Christensen* (Lyngby).

Trapping

The grids were coated by floating, face down (shiny side) for 5 minutes on a drop of diluted antisera. The antisera were diluted 1:1000 irrespective of the titer. The grids were rinsed with 30 consecutive drops of 0.1 M phosphate buffer pH 7.0, drained but not dried and immediately placed on a drop of the stored virus (not diluted). The incubation lasted 20 minutes at room temperature.

The procedure was finished by rinsing the grids with 30 consecutive drops of double distilled water followed by 6 drops of 2% aqued uranyl acetate (BHD).

Decoration

The rinsing of the grids after trapping, but before decoration, was carried out using 30 drops of phosphorous buffer and drained with filter paper, however, not allowed to dry. The grids were left

Table 1.

	Suspension	Year	Illustrations
Cymbidium mosaic virus (CyMV)	PTA	1972	Fig. 1A and 1B
Potato virus X (PVX)	AM	1972	2A and 2B
Tomato ringspot virus (TomRV)	PTA	1972	6
Cauliflower mosaic virus (CaMV)	PTA	1977	3A and 3B
Cocksfoot mottle virus (CFMV)	PTA	1975	4A and 4B
Tobacco ringspot virus (TobRV)	PTA	1972	5
Tobacco rattle virus (TRV)	PTA	1972	7A and 7B
Bean yellow mosaic (BYMV)	AM	1971	none
Narcissus yellow stripe virus (NYSV)	PTA	1972	none

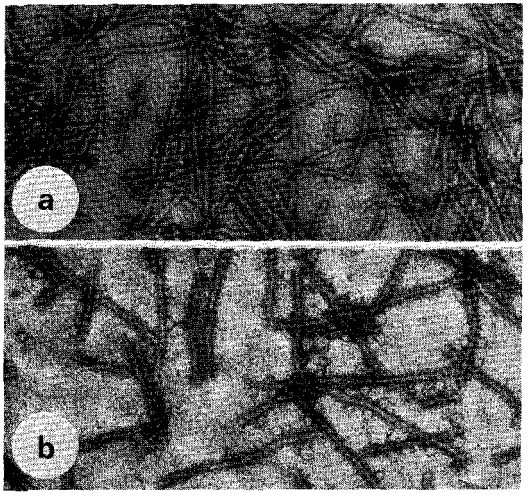

Fig. 1A. Cymbidium mosaic virus (CyMV) trapped.
1B. Same decorated. × 90.000.

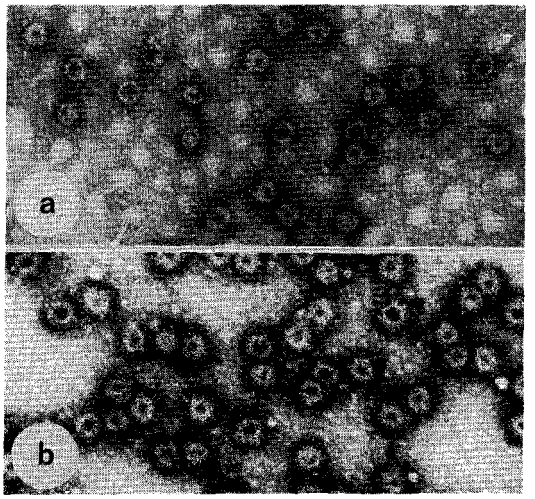

Fig. 3A. Cauliflower mosaic virus (CaMV) trapped.
3B. Same decorated. × 90.000.

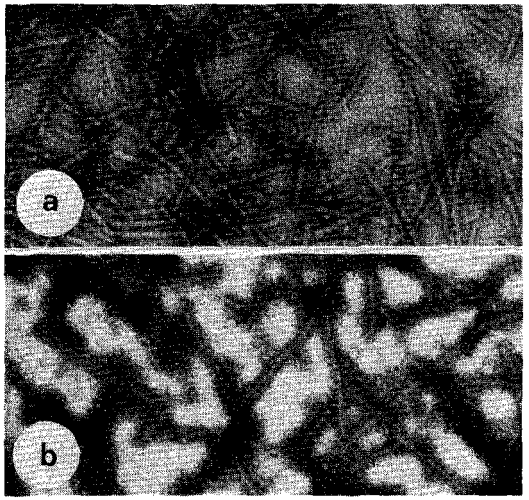

Fig. 2A. Potato virus X (PVX) trapped.
2B. Same decorated. × 90.000.

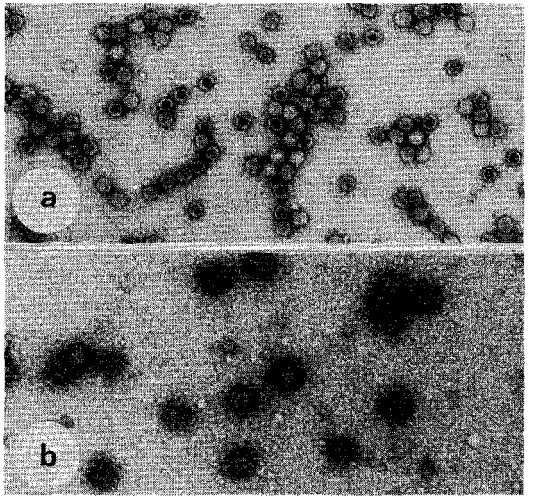

Fig. 4A. Cocksfoot mottle virus (CFMV) trapped.
4B. Same decorated. × 90.000.

20 minutes on a 10 μl drop of diluted antiserum (diluted 1:20–1:200) see later, rinsed with distilled water and stained with 6 drops of UA, as described before. The grids were decorated with diluted antiserum according to the amount of virus particles recognised at the trapped grids as follows

heavily loaded grids:
antiserum diluted 1:20–1:50.
medium loaded grids:
antiserum diluted 1:50–1:100.
light loaded grids:
antiserum diluted 1:100–1:200.

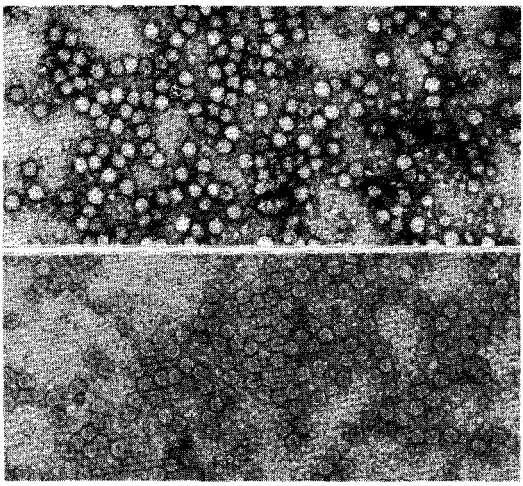

Fig. 5. Tobacco ringspot virus (TobRV) trapped.
6. Tomato ringspot virus (TomRV) trapped.
× 90.000.

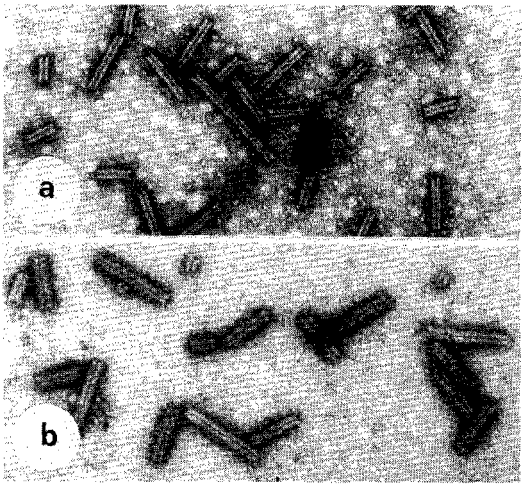

Fig. 7A. Tobacco rattle virus (TRV) trapped.
7B. Same decorated. × 90.000.

Results

Trapping

The viruses were easily trapped with the homolog antiserum on the grids. From illustrations Figs. 1–7 intact particles in varying amounts are shown in accordance to the information we have from the examination of the suspensions made years earlier.

The two last viruses on the list (Table 1) did not, for unknown reasons react in the ISEM test. The two samples were Bean yellow mosaic virus (BYMV) and Narcissus yellow stripe virus (NYMV). In accordance to this, no illustrations are shown.

Decoration

All virus particles detected by ISEM could be decorated. Examples are shown in Fig. 1B, 2B, 3B, 4B and 7B. It must also be mentioned, that there was a slight reaction between TomRV and TobRV in the decoration test.

Discusion

Some previous reports on the stability of viruses during storage (*Bos,* 1969; *Hollings & Stone,* 1970; *Regenmortel,* 1978; *Richter et al.,* 1978 a.o.) deal with this matter, but new problems have turned up working daily with antisera in different dilutions. The ISEM work requires regular control of diluted antisera to produce reproductive results.

The results of this experiment clearly show, that it is possible to store virus suspensions in an electron microscopic stain such as PTA, AM and also in UA. It is also shown that the serological activity is not affected by storage, even when stored under rather unfavourable conditions. The samples were not properly sealed and not looked at for many years. Bacterial growth was not observed in any of the samples.

This opens up possibilities of keeping a range of virus suspensions in storage. Such a »virusbank« could be used as a permanent control of the antisera and antisera dilutions used in daily ISEM work, in order to produce reliable test results and to enable the full interpretation of negative results also.

A new way of using this information, also opens up possibilities of assisting the developing countries with moderately or poorly equipped laboratories. If this method proves to be stable and practical in use, it should be possible to make cut squeeze or leaf dip samples in PTA on the spot in a developing country e.g. in the field or experi-

mental plot or breeding station etc. Then mail it as quickly as possible to the nearest EMLAB in the region, and get results by return. In this way it is possible to extend the radius of a electron microscope assistance to countries that have more need for capital investment for other purposes. In the years to come, experiments are planned to be carried out in order to evaluate the results of steady consignments between Copenhagen and a number of participants in app. 10 developing countries using this new preserving method for ISEM work over long distances. Some tests with virus samples from Mocambique and Zanzibar have already shown promissing results.

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