

ANALYSIS OF THE PHYSICAL BEHAVIOR OF VIRUSES USING THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS)

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ABSTRACT

A new method for virus analysis has been indicated for years. This situation has reached a new alarm status with the rapidly expanding number of new and emerging viruses that tax present detection methods. Adding the cost of current methods to analyze large numbers of samples and a clear and urgent situation has developed. Fortunately, a new means for detecting the present or absence of viruses has been developed that capitalizes on the fundamental physical properties associated with these tiny microbes. The Integrated Virus Detection System (IVDS) utilizes exquisite, but regular methods, to purify and concentrate samples for sizing and counting using the usual methods of Differential Mobility Analysis and Condensation Particle Counting. Thus a new method that is essentially a virus particle counter has been invented¹, patented² and demonstrated³ for a wide range of both enveloped and non-enveloped viruses. The invention is described and examples illustrated.

BACKGROUND AND INTRODUCTION

The Integrated Virus Detection System (IVDS) method for detecting viruses changes everything in respect to virus analysis. No culturing, no reagents, quick analysis, generic to all viruses. It counts whole virus particles, is suitable for counting all virus types and thus can also count unknown viruses or viruses we do not have other means to detect. The current instrument has been used to examine viruses separated from complex media⁴ with sensitivity that can approach 10⁴ viruses per milliliter⁵. Other virus

characteristics have been observed and calibrated, e.g. passage of viruses through filter membranes⁶ and exceptional survival in both extreme temperature and pH. It is the 4-nanometer separation of viruses of similar size⁷ that aids in this direct structural characterization of large number of viruses.

One of the first tests of this new technology was to compare it with other methods, such as Small Angle Neutron Scattering, SANS. Results were comparable⁸.

The second objective was to demonstrate the effectiveness of IVDS is counting viruses under a wide range of conditions. This is important as there are many inherent challenges to virus detection and analysis, among the primary is purification and concentration from the background material. This was accomplished for several viruses from many environmental samples, such as different soils including sand, drinking water, seawater and plants. Viruses were also analyzed from several other sources.

METHODS

MS2 Bacteriophage was first used as an example virus particle. This is standard practice in testing detection systems since it is environmentally stable and not infectious. MS2 is grown using standard procedures along with its host bacteria⁸. Other viruses were provided from other sources including animal tissue and fluids, and tissue culture media.

¹ Wick, C.H., et.al, *Quasi-Realtime Monitor for Airborne Viruses*, ERDEC-TR-459, January 1998

² Patent: Wick, C.H., Anderson, D., *System and Method for Detection, Identification and Monitoring of Sub-micron Sized Particles*, U.S. Patent 6,051,189, others pending.

³ Wick, C.H., McCubbin, P., *Characterization of the Integrated Virus Detection system (IVDS) Using MS-2 Bacteriophage*, ECBC TR-018, May 1999.

⁴ Wick, C.H., McCubbin, P., *Removing Complex Growth Media from MS-2 Bacteriophage Cultures*, ECBC-TR- 55, August 1999

⁵ Wick, C.H., McCubbin, P., *Characterization of Purified MS2 Bacteriophage by the Physical Counting*

Methodology used in the Integrated Virus Detection System (IVDS), *Toxicology Methods*, 9:245-252, 1999.

⁶ Wick, C.H., McCubbin, P., *Passage of MS2 Bacteriophage Through Various Molecular Weight Filters*, *Toxicology Methods*, 9:265-273, 1999.

⁷ Thomas, J.J., Bothner, B., Traina, J., Benner, H., and Siuzdak, G., *Electrospray ion mobility spectrometry of intact viruses*, *Spectroscopy* 18 (2004) 31-36.

⁸ Kuzmanovic, D., Elashivili, I., et. al. *Bacteriophage MS2: Molecular Weight and Spatial Distribution of the Protein and RNA Components by Small-Angle Neutron Scattering and Virus Counting*, *Structure* 11:1339-1348.

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INSTRUMENT OPERATION

A typical output from IVDS is given in Figure 1. Virus size and concentration are given as a graph with size in nanometers and concentration plotted. (1), a statistical analysis is reported (2) and a table of the actual counts per size bin (3). Statistical information includes mean size, total concentration, and a table of all the particles in the size range is prepared for each sample. In this manner the size, concentration and details of the analysis are presented. There is enough data in this chart to make a virus detection decision, to discriminate among samples and provide evidence for a preliminary identification.

Since this is a virus particle counting method, it is possible to count the samples longer (Figure 2) if the number of viruses is very low such as in seawater. Another method is to concentrate the sample to a smaller volume. Either method, or both can be used to detect low concentrations.

One feature of this counting method is that all the viruses in a sample ranging from 15 to over 400nm are all counted at the same time. In this way virus detection is completed in a couple of minutes for all the viruses in a sample and not just one at a time. This feature saves considerable time in making an assay of a large number of samples. Figure 3 is an example of multiple virus detection. The Kilham Rat Virus is clearly detected at it's expected 22.5nm size. Unexpected in the sample were the viruses at 34.6, 49.5 and 71nm that are common contaminants in tissue culture. This sample was prepared as close to the tissue culture media as possible, that is, the simplest means were used to prepare the sample and remove the background material that is smaller than the viruses. Additional preparation would remove nearly all the media and just the viruses would remain.

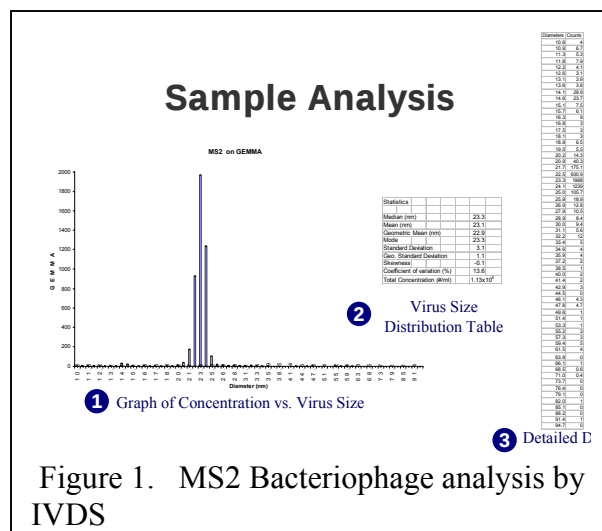


Figure 1. MS2 Bacteriophage analysis by IVDS

This demonstration is important since enveloped viruses can be more delicate and need to be analyzed closer to their natural source material.

CONCEPT OF OPERATION

Use as a first line detector to determine the presence or absence of a virus. We have many other methods that can be used to confirm or identify a virus and this process provides virus detection and confirmation by two different technologies. IVDS can be used to screen large number of samples since it is quick and can detect multiple viruses in the same sample. It can be an important means for detecting unexpected or unknown viruses.

