CHARACTERIZATION OF THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS): A NOVEL INSTRUMENT FOR VIRUS IDENTIFICATION AND ITS USE IN THE INITIAL CHARACTERIZATION OF A NEW MARINE VIRUS

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ABSTRACT

Detection and quantification of unknown or novel virus samples remains one of the great technical challenges in biological sciences. Here we report the characterization of an instrument, The Integrated Virus Detection System (IVDS) as an accurate and rapid means to size and quantify viral unknowns.

1. INTRODUCTION

Typically, virus size is determined by electron microscopy and virus concentration is measured by infectivity or optical density (O.D.) if the virus host and other physical features of the virus are known. Recent outbreaks of previously undescribed viral infections and the announcement of the creation of recombinant viruses have highlighted the need for a single instrument that can detect and identify viruses in solution about which nothing is known. Here, the Integrated Virus Detection System (IVDS), the only virus identification system designed for virus quantification and sizing, is analyzed in terms of the accuracy and reliability of its measurements (Wick. 1999). Specifically, to test the quantification ability of the IVDS instrument for virus sizing and counting two approaches where employed: 1) The accuracy of sizing by IVDS was determined using synthetic and biological sizing standards. 2) Evaluation of the concentration determination ability of IVDS was determined using the MS2 bacteriophage as a biomarker and Small Angle Neutron Scattering (SANS).

2. RESULTS AND DISCUSSION

2.1 SIZING ANALYSIS BY IVDS

The accuracy of sizing by IVDS was determined using synthetic and biological standards. Specifically, National Institute of Standards and Technology (NIST)-traceable size standard latex microspheres (data not shown) and the MS2 bacteriophage were both measured by IVDS. See Figures 1.

Figure 1.

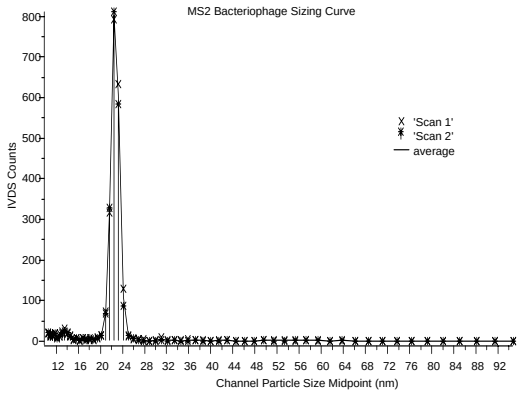

Table 1: Summary of MS2 Bacteriophage Particle Size Determinations

Sizing Method	Size (nm)	References
IVDS	23	This study
TEM	25	Overby, 1966
TEM	24	Sugiyama 1967
TEM	26	Straus 1963

Taken together, this data shows IVDS sizing measurements for the MS2 virus and NIST-traceable size standard latex microspheres are 23 and 43nm, respectively. This is in good agreement with the reported values above.

2.2 IVDS STANDARD CURVE

Also since the NIST-traceable microspheres are extremely well characterized, with respect to size as well as concentration, they were also used to generate a concentration standard curve for IVDS. See Figure 2.

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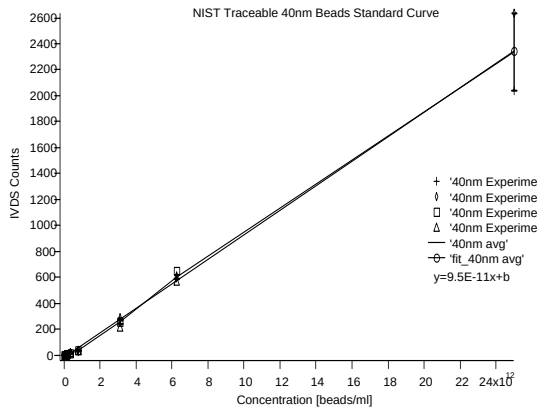

Figure 2.

The NIST microsphere standard concentration curve is linear and sufficient to generate an equation for the conversion of IVDS counts/ml to virus particles/ml.

2.3 EVALUATION OF IVDS CONCENTRATION DETERMINATION ABILITY

Testing of the concentration determination ability of IVDS was determined by measuring the concentration of the bacteriophage MS2 and then using this concentration to determine its molecular weight by SANS.

In general, SANS is a process where a neutron beam is passed through a sample and the resulting scattering pattern reveals information about the average size, shape, and orientation of the sample. Also, when the concentration of the sample is known, then molecular weight can be determined by SANS using absolute scaling (Krueger et. al 1998). Therefore, if IVDS accurately measures virus particle concentration then one should be able to accurately deduce the molecular weight of the virus using concentrations measured by IVDS. Since the molecular weight of MS2 has been determined by a number of different methods, MS2 is an ideal biomarker for use for testing of IVDS.

A stock of MS2 was measure by IVDS to get a concentration in IVDS counts/ml. The data was plotted on the NIST-Traceable concentration curve to convert those numbers into virus particles/ml. The same MS2 stock was also measured by optical density using a conventional spectrophotometry as a control since the use of optical density for virus concentration determination is well established. Finally the MS2 phage stock was subjected to SANS analysis and its molecular weight determined using both IVDS and optical density concentration measurements both methods were found to be in good agreement yielding resulting in molecular weights of 3.6×10^6. These results show that IVDS instrument is a rapid and accurate measure of virus sizing and concentration (which unlike other methods) requires no prior information about the unknown virus-containing sample.

3. MATERIALS AND METHODS*

For detailed materials and methods, see (Kuzmanovic et. al. 2003). *Certain commercial materials, instruments, and equipment are identified in this manuscript in order to specify the experimental procedure as completely as possible. In no case does such identification imply a recommendation or endorsement by the NIST nor does it imply that the materials, instruments, or equipment identified is necessarily the best available for the purpose.

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