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ECBC-TR-056

**CHARACTERIZATION OF MS2 BACTERIOPHAGE  
BY INTEGRATED VIRUS DETECTION SYSTEM (IVDS)  
PHYSICAL COUNTING METHODOLOGY**

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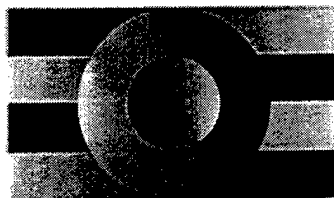
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13. ABSTRACT (Maximum 200 words) A new physically based methodology - The Integrated Virus Detection System (IVDS) - was used to characterize a high concentration, 10.2 mg protein/ml, sample preparation of MS2 Bacteriophage with a reported $10^{14}$ pfu/ml (DPM14) virus count in a common TNME buffer. Virus counts were made using the IVDS instrument following serial dilution. Results indicated virus counts of $1.5 \times 10^5$ for the neat sample (DPM14), followed by $6.5 \times 10^4$ viruses (DPM13), $1.2 \times 10^4$ viruses (DPM12), $9.3 \times 10^2$ viruses (DPM11), 88 viruses (DPM10), and 5 viruses (DPM9), respectively. Lower concentrations display a consistent multiplier and were consistent with target dilutions. Increases in virus concentration appear to decrease the multiplier. Variation is considered to be due to aggregation. Results demonstrate a consistent and simple-to-use methodology. Results further indicate that the IVDS instrument can be used for characterization of other virus preparations with equal ease and similar results.			
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## **PREFACE**

The work described in this report was performed as part of a Defense Advanced Research Projects Agency (DARPA) project. This work was started in March 1998 and was completed in September 1998.

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# Characterization of MS2 Bacteriophage by Integrated Virus Detection System (IVDS) Physical Counting Methodology

## 1. Introduction

The detection and analysis of viruses has been the goal of science for many years, following the first evidence that a new type of microorganism was responsible for diseases in both man and animals. These viruses were smaller than bacteria and thus presented the first challenge. Their small size made classification of these new microbes difficult and the field of virology was advanced by biochemical techniques rather than by direct examination. In more recent times and advancements in electronmicroscopy helped this problem and much information has been reported on the physical features of more than 21 virus families. These historic techniques are, however, time consuming, and require special knowledge, specialized chemicals or reagents and techniques to be successful. It was found possible based on the physical characteristics of viruses to count the individual viruses directly in a new and dramatic way using the Integrated Virus Detection System (IVDS)<sup>1,2</sup> instrument, which uses easily obtained materials and simple to operate techniques.

This new instrument was used to analyze and characterize a sample of MS2 bacteriophage provided by the Life Sciences Division at Dugway Proving Ground (DPG). This sample was 2 ml of purified MS2 bacteriophage at a concentration  $1 \times 10^{14}$  plaque forming units (pfu)/ml or 10.2 mg protein/ml. This highly purified sample is from Lot #98110.

The MS2 sample was analyzed using the IVDS instrument or more directly the Gas-phase Electrophoretic Mobility Molecular Analyzer (GEMMA) detector which is one stage of the IVDS instrument. The GEMMA detector consists of an electrospray unit to inject samples into the detector, a Differential Mobility Analyzer and a Condensate Particle Counter. A complete description of the IVDS system, including the GEMMA detector, can be found in the report *Virus Detection: Limits and Strategies*.<sup>3</sup>

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<sup>1</sup> Patent Pending on the IVDS technology.

<sup>2</sup> Wick, C.H., Anderson, D.M., and McCubbin, P.E., *Characterization of the Integrated Virus Detection System (IVDS) Using MS-2 Bacteriophage*, ECBC-TR-018, U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD, May 1999.

<sup>3</sup> Wick, C.H., Yeh, H.R., Carlon, H.R., and Anderson, D., *Virus Detection: Limits and Strategies*, ERDEC-TR-453, December 1997.

## 2. Laboratory Testing of MS2 Bacteriophage

### 2.1 Results

The high purity MS2 sample, with  $1 \times 10^{14}$  pfu/ml (hereafter described as DPM14) was analyzed using the GEMMA virus detector. The sample of DPM14 was placed neat into the GEMMA analyzer and the results are shown in Figure 1. The graph shows a very high virus count (over 150,000 counts) as well as other features. MS2 is nominally 24-26nm in size and this is illustrated in Figure 1. In fact, the sample as received was difficult to aspirate through the capillary delivery system in the GEMMA.

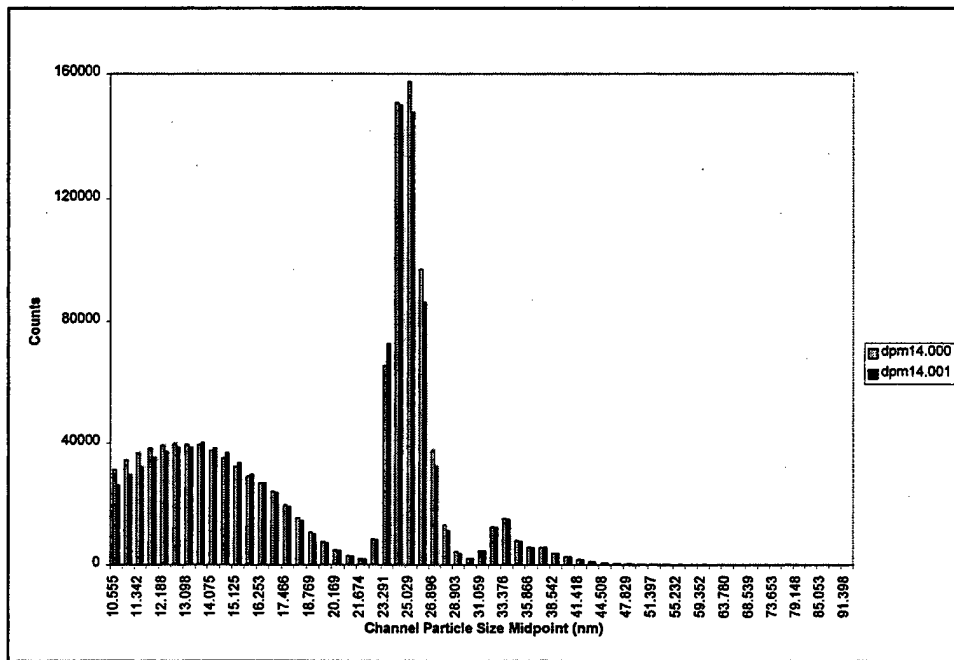
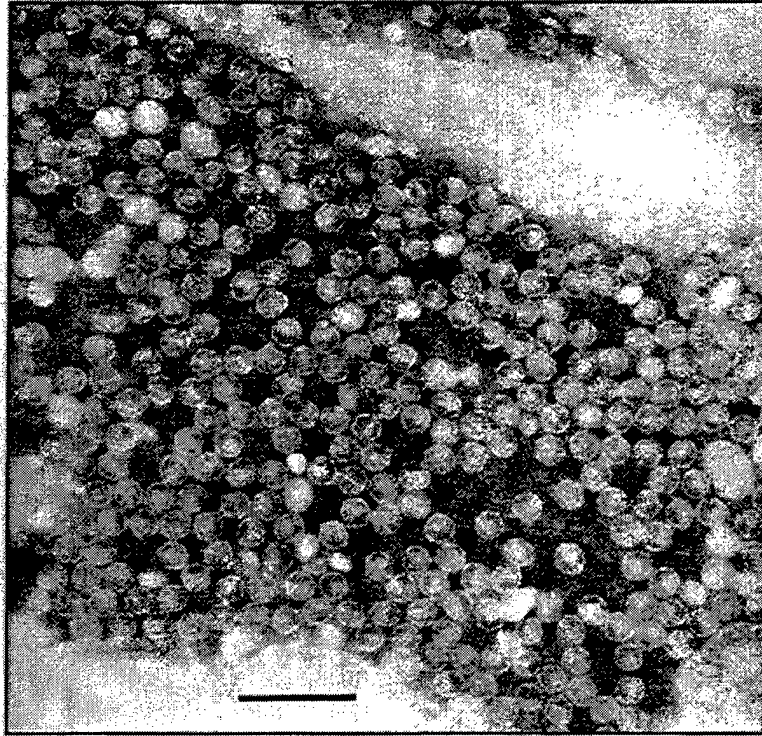


Figure 1 MS2  $1 \times 10^{14}$  pfu/ml, DPG Lot #98110

The size range of 24-26 nm is the expected size for a MS2 bacteriophage, as shown in Figure 2 in a micrograph by Dr. Hans Ackermann.<sup>4</sup>

<sup>4</sup> Micrograph located at <http://life.anu.edu.au/viruses/welcome.htm>.



**Figure 2 Micrograph of MS2 bacteriophage (bar represents 100 nm)**

When the difficulty of sampling the neat MS2 sample became apparent, the sample DPM14 was then serially diluted to produce a number of lower concentration samples. That is, an aliquot of DPM14 was diluted 10 fold to produce a sample of MS2 at a concentration of  $1 \times 10^{13}$  pfu/ml. This sample was named DPM13. The dilutions were all made with a 0.02M solution of ammonium acetate (pH~10), which is required for the electrospray unit. The pH was adjusted to keep the virus from breaking down into its component subunits. Sample DPM13 was then diluted 10 fold, and likewise for the following dilutions. Table 1 lists the samples that were produced by serially dilution of the original sample.

**Table 1 Serial Dilution Samples of MS2**

DPM13	$1 \times 10^{13}$ pfu/ml
DPM12	$1 \times 10^{12}$ pfu/ml
DPM11	$1 \times 10^{11}$ pfu/ml
DPM10	$1 \times 10^{10}$ pfu/ml
DPM9	$1 \times 10^9$ pfu/ml
DPM8	$1 \times 10^8$ pfu/ml

Figures 3-8 show the resultant GEMMA analysis of the serially diluted MS2 samples.

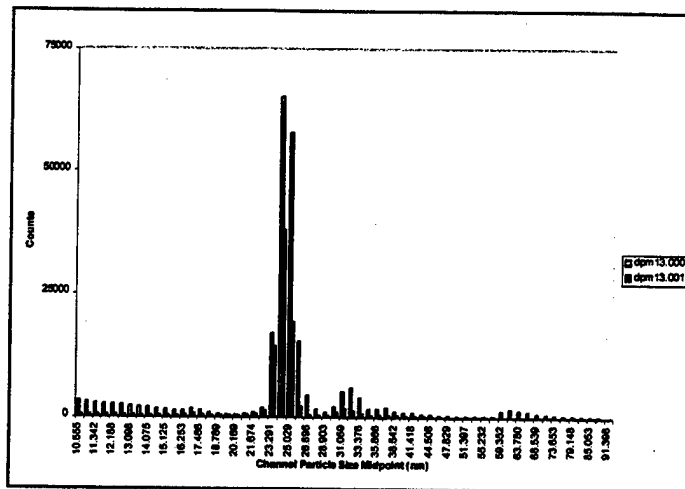


Figure 3 MS2  $1 \times 10^{13}$  pfu/ml, DPG Lot #98110

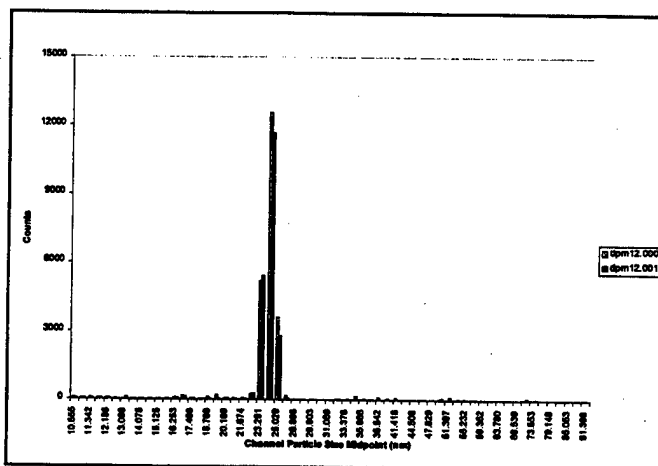


Figure 4 MS2  $1 \times 10^{12}$  pfu/ml, DPG Lot #98110

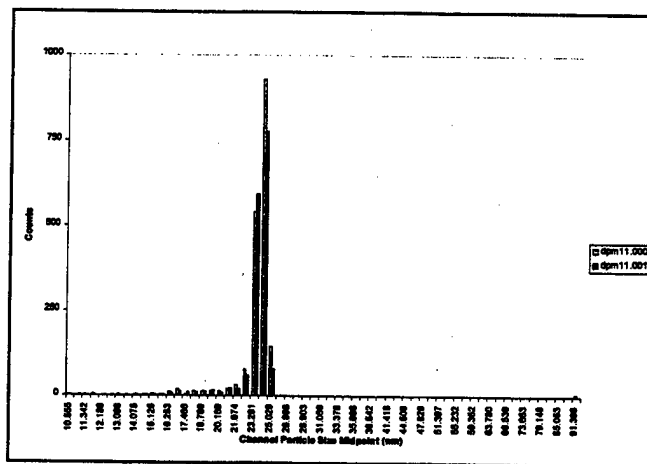


Figure 5 MS2  $1 \times 10^{11}$  pfu/ml, DPG Lot #98110

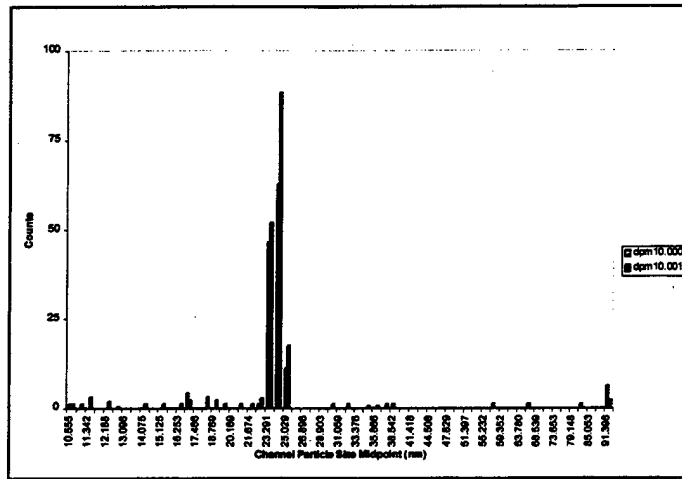
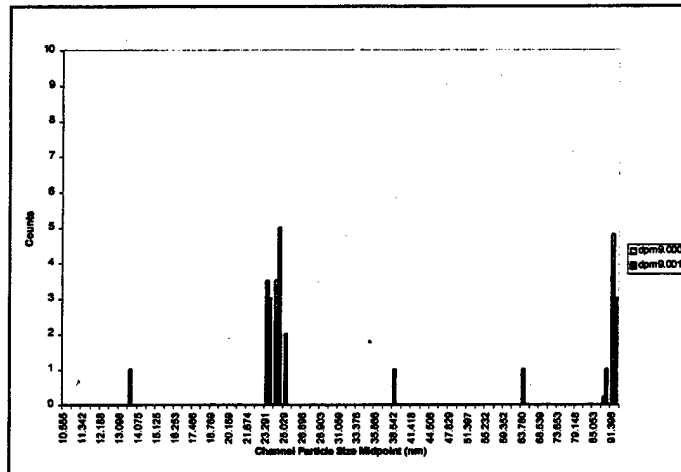


Figure 6 MS2  $1 \times 10^{10}$  pfu/ml, DPG Lot #98110



The counts for the serial dilutions were tabulated and are shown in Table 2.

**Table 2 IVDS Physical Counts for MS2 Samples**

MS2 Sample	Counts in Size Range				
	25.946 nm	25.029 nm	24.144 nm	23.291 nm	22.468 nm
DPM8			1		
DPM9		2	5	3	
DPM10		17	88	52	
DPM11		146	929	541	78
DPM12	148	3613	12582	5174	255
DPM13	15216	57624	65021	16893	1664
DPM14	96995	157461	150886	65389	8347

## 2.2 Analysis

The GEMMA detector easily detects MS2 bacteriophage. The virus is consistently detected in the range of 22 to 26 nm. The GEMMA scans also show very low backgrounds away from the MS2 peaks. The action of serially diluting the MS2 did not affect the stability of the bacteriophage in solution. In fact, the addition of ammonium acetate buffer to produce dilutions reduced the background counts. The GEMMA scans of buffer solutions show very low counts, as ammonium acetate is nearly invisible to the detector.

The count rates for the various concentrations of MS2 were tabulated in Table 3. A comparison of the multiplication factor from sample to sample was also tabulated in the table. The lower concentrations display a fairly consistent multiplier and are consistent with the target dilutions. As the concentrations increase, the multiplier appears to decrease in magnitude. As was noted in Section 1.1, the as received sample, DPM14, was difficult to aspirate into the GEMMA detector. This sample is very concentrated and this appears to interfere with the analysis. The reduction in the multiplier may be caused by the agglomeration of particles as they flow through the Condensate Particle Counter (CPC) in the GEMMA unit. This agglomeration would lower the amount of particles counted and reduce the multiplier. It would appear that a count rate over 100,000 counts in a few adjacent channels, with a virus in this size range of 25 nm, is approaching an upper limit to concentrations that can be analyzed in the detector. This is easily remedied by simply diluting a sample to less than 100,000 counts in adjacent channels.

**Table 3 Numerical Analysis of MS2 Peak Count Information**

MS2 Sample	Sum of size range	Multiplier from sample to sample
DPM8	1	-
DPM9	10	10.0
DPM10	157	15.7
DPM11	1694	10.8
DPM12	21772	12.9
DPM13	156418	7.2
DPM14	479078	3.1

The actual sensitivity of the GEMMA detector was not in question in this study. The presented solution to the detector can be further concentrated to allow for the analysis of samples that appear to be too dilute. The sample DPM8 could be concentrated from one ml, the original volume, to 10  $\mu$ l. This would then present the GEMMA detector with a sample that would generate a graph with ~100 counts in a scan. The number of viruses that can be detected by the GEMMA is very low, on the order of 10 viruses, and therefore the ability to detect viruses is only a function of the presented solution concentration. A further example was a simple experiment where a few thousand viruses were measured into 500 ml of water. The water sample was concentrated through the Ultrafilter unit and nearly 800 viruses were counted by the GEMMA. The limiting factor for analysis is the ability to further concentrate a liquid solution while still being able to effectively handle the solution without losing it due the handling problems associated with tiny volumes.

### **3. Conclusions**

The sample of MS2 bacteriophage received from the Life Sciences Division at Dugway Proving Ground was a very pure and concentrated sample. No other viruses were detected. The sample responded well to serial dilutions and was stable in the ammonium acetate buffer. This technique is a simple method to test the purity of any virus preparation since the IVDS instrument is not limited to any particular virus.