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DETERMINATION OF MS2 BACTERIOPHAGE STABILITY AT LOW pH USING THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS)

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14. ABSTRACT This report describes testing of the survivability of MS2 virus under harsh conditions using the Integrated Virus Detection System (IVDS). In this study, the MS2 was subjected to a highly acidic and oxidative environment. Judging by the concentration of the virus particles, the virus survived 66 hr in 0.1N HNO ₃ solution without any signs of diminution in the number of virus particles. This suggests that viruses might be able to survive such harsh environmental conditions.																	
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PREFACE

The work described in this report was started in June 2004 and completed in December 2004.

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DETERMINATION OF MS2 BACTERIOPHAGE STABILITY AT LOW pH USING THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS)

1. INTRODUCTION

The stability of viruses under different environmental conditions has always been a problem for microbiologists. Measuring this stability requires subjecting the virus to a harsh environment and monitoring the decay of the number concentration of virus particles with time. Until recently, measuring this stability had been exceedingly difficult. However, a new device – the Integrated Virus Detection System (IVDS), which can characterize and measure the number concentration of viruses, has been developed at the U.S. Army Edgewood Chemical Biological Center (ECBC). The IVDS relies on measuring physical characteristics (size) of the virus instead of bacteriological means. This allows us to measure the number concentration of virus particles quickly. The IVDS is described in ECBC-TR-018.¹

This report is the second in a series of reports that describes studies on the survivability of MS2 bacteriophage under different environmental condition. This report discusses the fate of MS2 at low pH. The first report dealt with the fate of MS2 at high pH.²

2. EXPERIMENTAL PROCEDURES

A sample of MS2 stock solution (2 mL) was obtained from Dr. Deborah Kuzmanovic, National Institute of Standards and Technology (NIST), Gaithersburg, MD. The stock solution was diluted with 100 mL of distilled water and filtered by the Ultra Filtration (UF) subsystem of the IVDS using 100K-Da filters (for details on the IVDS and its different subsystems, the reader is referred to ECBC-TR-018 and ECBC-TR-463.³

The filtration system removes any material with a molecular weight smaller than the filtration system is set for (in this case, 100K-Da), such as growth media, salt molecules, and proteins from the solution and leaves a concentrated virus solution. The concentrated MS2 solution was added to 23 mL of 20 mM ammonium acetate solution. The ammonium acetate is needed to increase the conductivity of the solution to allow it to be injected into the test module of the IVDS. The MS2 solution was concentrated again by the UF subsystem to a total of

¹ Wick, C.H.; Anderson, D.M.; and McCubbin, P.E. Characterization of the Integrated Virus Detection System (IVDS) using MS2 Bacteriophage; ECBC-TR-018, U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 1999; UNCLASSIFIED Report (AD-A364 117).

² Wick, C.H.; Elashvili, I.; McCubbin, P.E.; and Birenzvice, A. Determination of MS2 Bacteriophage Stability at High pH using the Integrated Virus Detection System (IVDS); ECBC-TR-472; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 2006; UNCLASSIFIED Report.

³ Wick, C.H.; McCubbin, P.E.; and Birenzvice, A. Detection and Identification of Viruses using the Integrated Virus Detection System (IVDS); ECBC-TR-463; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, November 2005.

2.5 mL of clean solution. The bacteriophage solution was then subjected to low pH to determine the survival rate.

For low pH testing, 10 μl of the MS2 stock solution was added to 90 μl 0.1 N nitric acid (HNO_3). The resultant solution had a pH of 1.4. Prior to scanning by the IVDS, the acidic solution was neutralized by adding a buffer solution comprised of 5.3 μl of 0.1 N ammonium hydroxide (NH_4OH) and 84.7 μl of 20 mM solution of ammonium acetate (pH = 7.05). The low pH MS2 solution was neutralized a few minutes after subjecting the MS2 to the low pH and again after 66 hr.

3. RESULTS AND DISCUSSIONS

The original stock solution of MS2 (a description of the virus and its breakdown material was discussed in ECBC-TR-463),³ was diluted 1:10 with a solution of 20 mM ammonium acetate and scanned by the IVDS. The scan results are shown in Figure 1. The counts in Figure 1 were numerically divided by a factor of 10 to graphically compare the stock solution with results after subjecting the MS2 to low pH environment.

Figures 2 and 3 represent the IVDS scan results immediately after it was subjected to the acidic conditions and again after 66 hr, respectively. As can be seen, the MS2 particles counts are roughly the same indicating that the MS2 can survive intact at low pH for many hours. The results are also tabulated in the table herein.

Figure 4 shows the IVDS's scan results for a particle range of about 11 to 20 nm, where we can expect to find degradation products of MS2. As can be seen, the count at this size range remains fairly consistent. This is further confirmation that MS2 is stable under strong acidic conditions as low as 1.4 pH. We also need to point out that HNO_3 is considered to be a strong oxidative solution, indicating that some viruses can survive such harsh environments.

4. CONCLUSIONS AND RECOMMENDATIONS

This study shows that certain viruses can survive harsh environments (e.g., low pH and oxidative environments). The results of this study are important because H_2O_2 vapors are being considered as means to decontaminate building interiors after biological terrorist attacks. Because viruses can be used as terrorist weapons, we recommend that the fate of viruses considered as potential biological weapons be examined. At this point, we need to emphasize that in the study described herein, we only checked for maintenance of the structural integrity of virus particles. We did not test for virus viability.

MS2 Low pH Stability Baseline

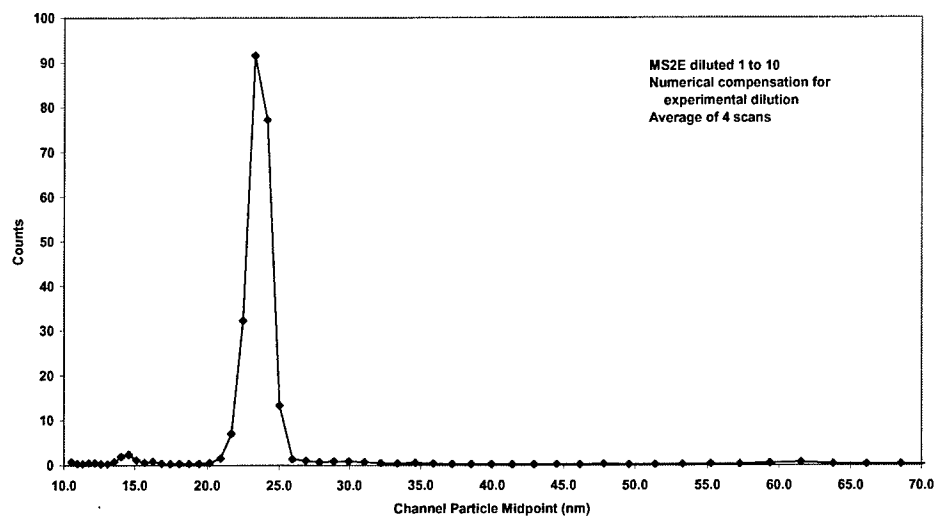


Figure 1. MS2 Sample Baseline Count

MS2 Low pH Stability

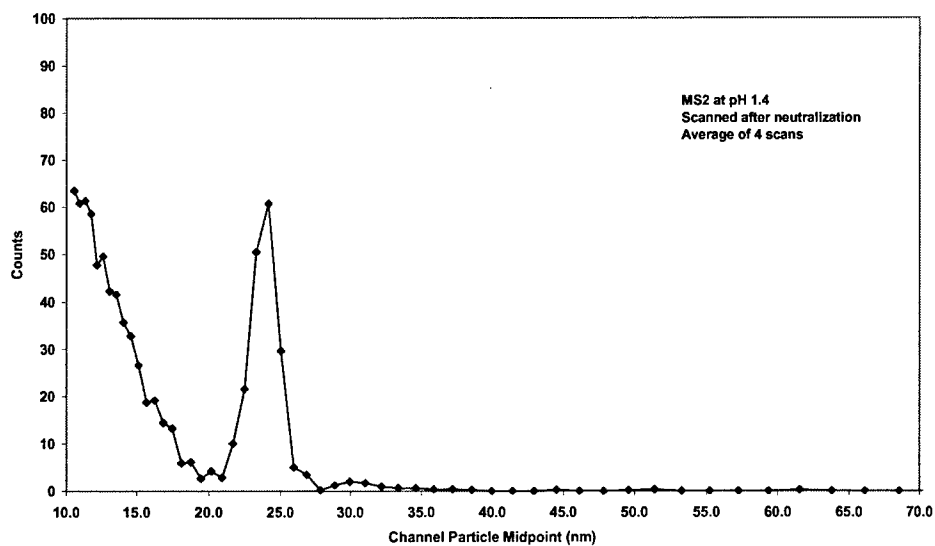


Figure 2. IVDS's Scan Immediately after Adding the Low pH Solution

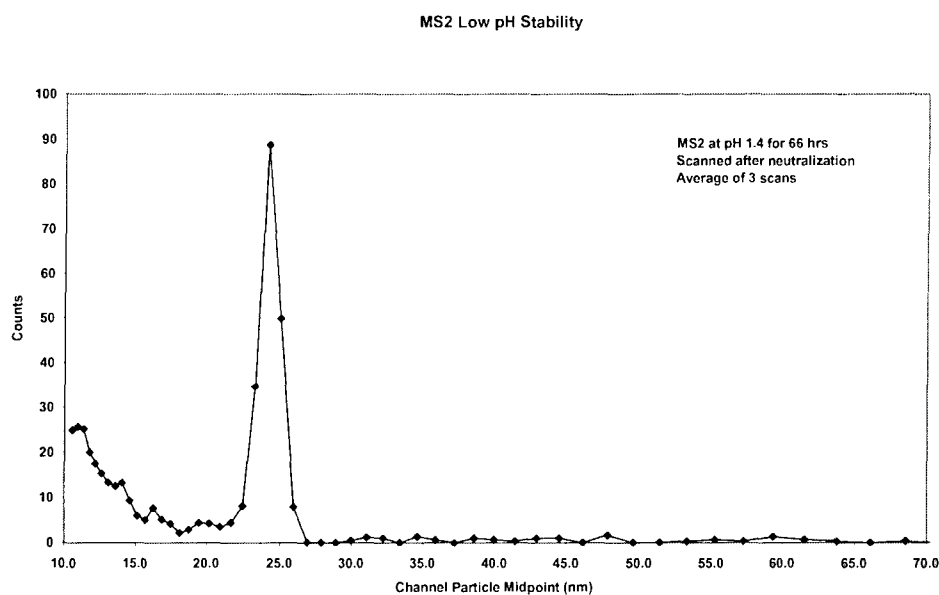
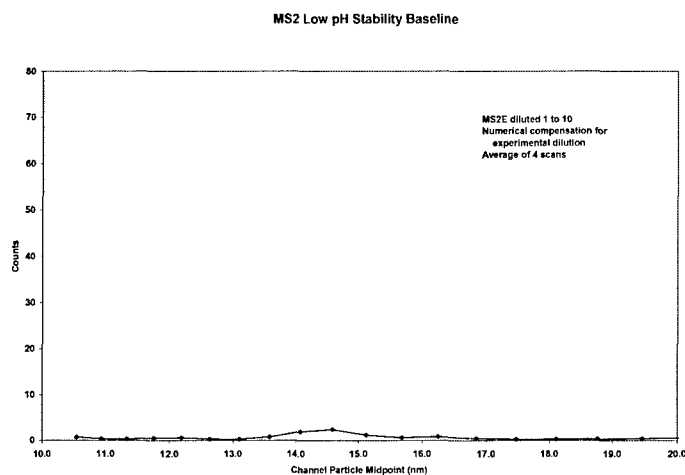


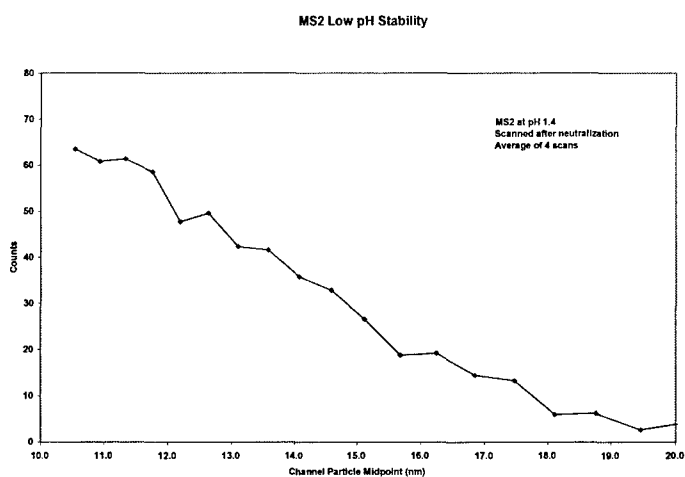
Figure 3. IVDS's Scan of MS2 at Low pH after 66 hr

Average Particle Count of MS2 Scans

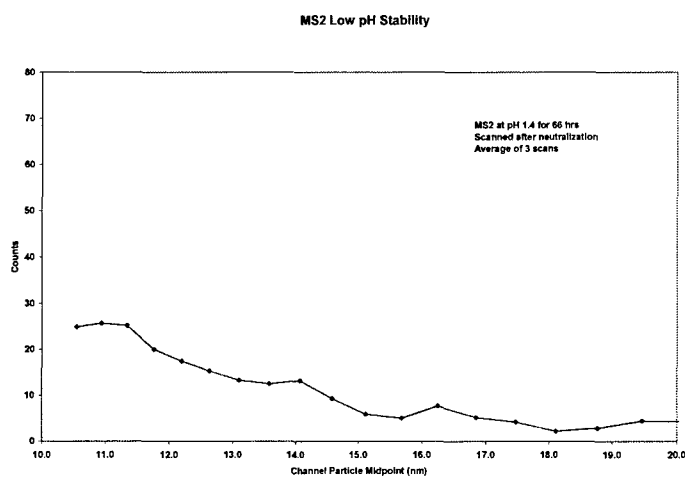
	IVDS counts at size range of 10.55 to 19.46 nm	IVDS counts at size range of 20.17 to 29.96 nm
Baseline	12 ± 0.6	229 ± 32
Initial sample	601 ± 21	192 ± 21
66 hr at pH 1.4	214 ± 8	202 ± 28



a. MS2 baseline scan



b. immediately after adding low pH solution



c. 66 hr at low pH

Figure 4. IVDS's Scan for MS2 Breakdown Products at Low pH