



## **PRELIMINARY RESEARCH REPORT:**

# **Comparison of Three Methods of Detecting Residual Microorganisms on a Cleaned/Disinfected Surface**

The Toxics Use Reduction Institute  
Academic Research Program

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# **Comparison of Three Methods of Detecting Residual Microorganisms on a Cleaned/Disinfected Surface**

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## Introduction

Traditional disinfectants are harsh chemicals that are well known to have harmful health effects. For example, bleach (sodium hypochlorite) and quaternary ammoniums are well known respiratory irritants and have been linked to asthma, including occupational asthma in professional cleaning personnel and others who frequently use disinfectants, such as health-care workers. Safer alternatives to traditional disinfectants are needed. Commercial “green” products are available as well as do-it-yourself recipes that claim disinfection. However there is little independent data supporting disinfection claims. In order to collect the robust amount of data that is needed to scientifically determine the effectiveness of alternative disinfection methods, a rapid method of detecting residual microorganisms on a surface must be available for field use. The traditional methods of detection are culture based (contact agar plates or swabs that are then cultured). While culture methods are sensitive and accurate, they are time-consuming, costly, and require expertise. Two newer rapid methods of detection are available as hand-held devices designed to be used on site. Each involves using a special swab to sample the area in question, and each provides a reading with a small hand-held meter. Neither of these devices have been fully characterized to ensure proper use and interpretation.

The first approach is based on bioluminescent detection of adenosine triphosphate (ATP). ATP is present in all biological cells, including human and plant cells, as well as bacteria. Several ATP devices are available on the market however this study used the Hygiena SystemSure Plus (Hygiena, Camarillo, CA). The meter results are reported as relative light units (RLUs) and interpreted as dirty ( $\geq 30$  RLU), not adequately cleaned (11 – 29 RLU), or clean ( $\leq 10$  RLU).



The second approach detects specific enzymes with a fluorometric method. This device is also rapid and easy to use, and has the advantage of greater specificity in detection as different kits are available to detect enzymes that are specific to certain bacterial species or multiple species.

We evaluated the devices for 1) sensitivity across the growth curve in comparison to culture for *Escherichia coli* and *Staphylococcus aureus*, 2) with-in run and between-run precision, and 3) a pilot field test.

## Fluorometer Test Results

We performed limited testing with the fluorometric enzyme device and found the results to be inconclusive. Full testing was not performed with this device.

## SystemSure Sensitivity Across the Growth Curve

Sensitivity was determined for *E. coli* and *S. aureus* during lag, log, stationary, and death phases of the growth curve. Bacteria are cultured in a nutrient medium for 3 days. For the first 12 hours, aliquots are removed every 2 hours, diluted and tested. After 12 hours, aliquots are removed at 24, 48, and 72 hours. At each time point, dilutions are tested for ATP reading and plated for colony counts. The plates are incubated overnight and colonies counted the next day. A reading of  $\leq 10$  RLUs (clean) was used as the lower limit of detection for the meter, and compared to the number of colonies present at that growth stage. It was determined that sensitivity of this device is variable along the different stages of bacterial growth (see Table 1), and at no point in the growth curve does a “clean” reading guarantee no living bacteria are present. Variability was also seen between the two organisms, as their rates of growth were different. In addition, readings above 30 RLU (dirty) were not found to be linear when compared to serial dilutions of the culture.

Table 1. Sensitivity during lag, log, stationary, and death phases for *E. coli* and *S. aureus*. Minimum colony forming units detected.

	Lag Phase (CFU/mL)	Log Phase (CFU/mL)	Stationary Phase (CFU/mL)	Death Phase (CFU/mL)
<i>E. coli</i>	217,500	24,000	286,000	1,010,000
<i>S. aureus</i>	111,500	1,710	1,530,000	1,110,000

## Precision

Between-run precision was determined with positive control, and within-run precision was determined with control, lag and log phase cultures. In each case, 10 replicates of the same control or culture was run. For between-run precision, the replicates were tested on different days. For within-run precision, all replicates were run at the same time. Both between-run and within-run precision of the device was adequate, with all replicates falling within a 95% confidence level.

## Pilot Field Test

A pilot field study was conducted in a restroom in a UMass Lowell campus building. Cultures by contact plate and ATP readings were taken before and after cleaning on 4 surfaces (toilet seat, floor by toilet, stall door, faucet handle). The field pilot showed a decrease in meter readings for

most surfaces post cleaning, however the results were inconsistent. Further evaluation of the meter in the field will need to be performed, along with consideration of irregular surfaces such as faucet handles.

### **Application of These Results to Evaluation of Safer Disinfectants**

These results demonstrate that the SystemSure can be used in the field to evaluate pre- and post-cleaning efficacy, but it does have limitations. Due to the lack of linearity, especially in the higher range (readings above 1000 had a standard deviation of 400), caution must be used when interpreting a decrease in RLUs. However, when interpreted qualitatively (clean, not adequately cleaned, dirty) comparisons between traditional and alternative disinfection methods can be made using the SystemSure.

### **Conclusion and Future Work**

Characterization of the meter was a goal of this study to determine appropriate use of the device. Current users may not be aware of the limitations of the meter and therefore may not interpret the readings correctly. The meter is limited by its relative insensitivity at low levels of bacterial ATP in the lab setting and lack of linearity at higher readings. The precision of the instrument is adequate. However, the meter can be used to demonstrate post-cleaning reduction of biological soil, with or without bacteria. Further evaluations will include: additional ATP bioluminescence meters, interference testing, artificial soils with and without bacteria, and further field testing.