

ABSTRACT

To evaluate the performance of safer (green) approaches to disinfection, rapid methods of detecting residual microorganisms on a surface are needed. Contact agar plates are sensitive and accurate, but time-consuming, costly, and require expertise. New devices based on bioluminescent detection of ATP have been marketed as rapid alternatives for detecting residual microorganisms. These devices have not been fully evaluated to ensure proper use and interpretation.

We evaluated the Hygiena SystemSure Plus (Hygiena, Camarillo, CA) for sensitivity across the growth curve and precision in comparison to culture for *Escherichia coli* (ATCC 21214) and *Staphylococcus aureus* (ATCC 6538). Sensitivity was determined for *E. coli* and *S. aureus* during lag, log, stationary, and death phases of the growth curve. Between-run precision was determined with positive control, and within-run precision was determined with control, lag and log phase cultures.

Sensitivity varied with the stages of the growth curve. Sensitivity for *E. coli* was 217,500, 24,000, 286,000, and 1,010,000 CFU/mL for lag, log, stationary, and death phases respectively. Sensitivity for *S. aureus* was 111,500, 1,710, 1,530,000, and 1,110,000 CFU/mL for the same phases.

Between-run precision (CV) for the control was 16%. Within-run precisions (CV) were: positive control 12%, lag phase *E. coli* 63%, log phase *E. coli* 19%, lag phase *S. aureus* 58%, and log phase *S. aureus* 18%.

ATP meter sensitivity is dependent on bacterial growth stage; meter users need to take this into account. Precision of device is adequate with both organisms. Future work will include a field pilot study.

SIGNIFICANCE

- The rapid detection of residual microorganisms on surfaces is needed to ensure cleanliness is achieved from the cleaning process. This is important to human health as a measure for infection control.
- Harsh chemicals have proven to be effective in eliminating infectious diseases on various surfaces; however, there are harmful side effects for human health as well as negative environmental impacts from these products.
- Proof of effectiveness of green or low toxicity products by rapid meter detection can help the cleaning industry to eliminate traditional toxic products and implement use of safer alternative choices.

PROCEDURE

Growth Curve: Organisms used were *E. coli* and *S. aureus*, each with their own growth curve.

Day One: Nutrient broth was inoculated with a single colony of bacteria for overnight growth at 35 ° C.

Day Two: 1 ml of overnight inoculum was introduced to 100ml of nutrient broth and sampled for time zero. Additional samples were tested in increments of 2 hrs for a total of 12 hours. At each time point, serial 10-fold dilutions in PBS were spread plated and tested on the meter.

Day Three: 24 hour time point was sampled. Colony counts from Day two spread plates were performed.

Day Four: 48 hour time point was sampled. Colony counts from Day three spread plates were performed.

Precision Data Collection:

- Within-run precision: Positive control, log and lag phase cultures were tested for precision 10 times in a row on the same day. Mean, standard deviation, and coefficient of variation were calculated.
- Between-run precision: Positive control was read 10 times on 10 different days. Mean, standard deviation, and coefficient of variation were calculated.

THE ATP METER

Hygiena SystemSure Plus (Hygiena, Camarillo, CA)

- Results are reported as relative light units (RLU) and interpreted as dirty (≥ 30), not adequately cleaned ($11 - 29$), or clean (≤ 10).



- The image below is showing an RLU of 3; that reading would be reported as clean.



RESULTS

Growth curves of *E. coli* and *S. aureus*: Culture plate growth and ATP meter reading

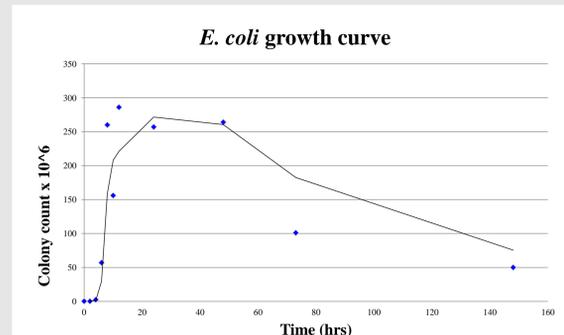


Figure 1. *E. coli* growth curve with colony counts

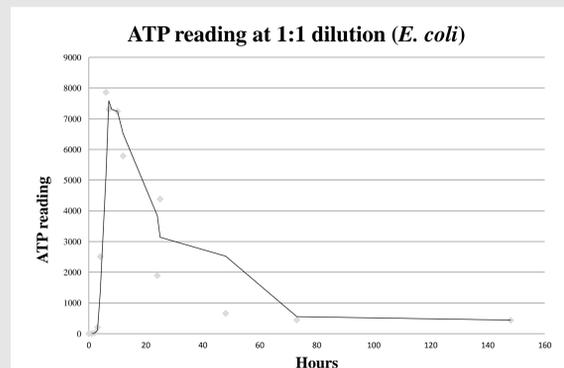


Figure 2. *E. coli* growth curve read on ATP readings

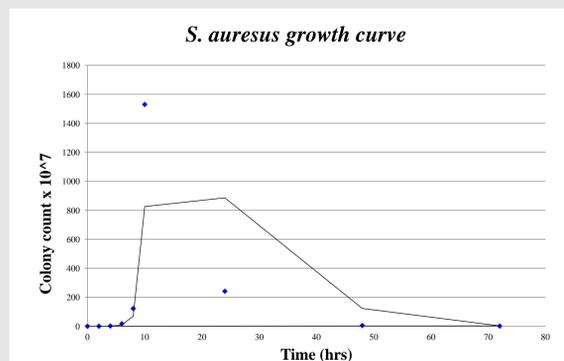


Figure 3. *S. aureus* growth curve with colony counts

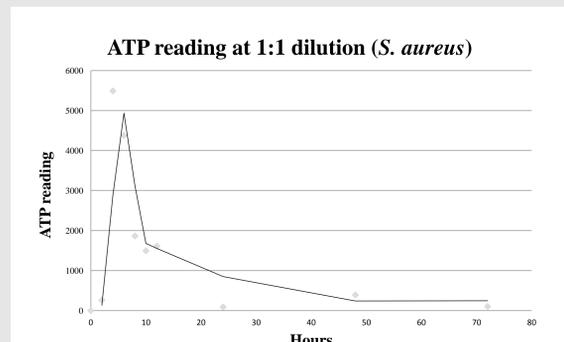


Figure 4. *S. aureus* growth curve with ATP reading

RESULTS

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

Table 1. Sensitivity of ATP Meter during Lag, Log, Stationary, and Death phases of the Growth Curve for *E. coli* and *S. aureus*. Colony count at which ATP meter read ≤ 10 .

Sample	Mean	Standard Deviation	Number of Replicates outside ± 2 SD	CV %
Positive Control	274	34	1	12
<i>E. coli</i> Lag Phase	1.3	0.82	1	63
<i>E. coli</i> Log Phase	2173	404	0	19
<i>S. aureus</i> Lag Phase	2	1.16	1	58
<i>S. aureus</i> Log Phase	5535	970	3	18

Table 2. Within-Run Precision: All samples were run 10 times in a row on the same day.

Sample	Mean	Standard Deviation	Number of Replicates outside ± 2 SD	CV %
Positive Control	232	36	0	16%

Table 3. Between-Run Precision: All samples were run 10 times on 10 different days.

CONCLUSION

Sensitivity Data:

- The CFU/mL at the dilution at which the meter read "clean" (≤ 10 RLU) indicated the quantity of bacteria missed by the meter reading.
- S. aureus* meter sensitivity needs to be repeated because of lower/equal sensitivity in stationary phase than death phase.

Precision Data:

- The meter demonstrated good precision with most readings within a 95% confidence limit.
- Precision data doesn't support linear interpretation of meter results, especially at higher numbers.

Future Directions:

- Pilot field study including:
 - Lab testing of soiled surface pre- and post-cleaning
 - Testing in real world setting pre- and post-cleaning process