The Comparison of Four Bioluminometers and Their Swab Kits for Instant Hygiene Monitoring and Detection of Microorganisms in the Brewery

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Four luminometers and their swab units were evaluated for detecting ATP by surface swabbing. Testing included pipetting known quantities of ATP directly onto the swabs; pipetting known levels of bacteria and yeast directly onto the swabs and swabbing samples of bacteria and yeast from a surface. None of these instruments and swab detection kits provided consistent, reproducible detection of ATP standards or ATP from microorganisms even at high concentrations. All of the swab kits/instruments showed poor linearity in measuring known quantities of ATP and showed high variability in ATP readings with replicate swabs containing identical concentrations of microorganisms. Since good linearity and reproducibility could be obtained using a liquid sample assay of ATP standards without swabs, it is suggested that the swab method itself may be unreliable. ATP may not be effectively released from microorganisms on swabs; ATP may adsorb to the swab interfering with detection and/or the swab might block light transmission. Swabs of bacterial/yeast suspensions dried on a sterilized surface, provided the most inconsistent ATP readings and lacked linearity. A reason for the poor detection of microbial ATP by surface swabbing could be the inability to pick up microorganisms effectively.

Key Words: *ATP, bioluminometers, microorganisms, brewery, hygiene, swab.*

INTRODUCTION

All living cells contain ATP (adenosine triphosphate) which can be extracted and assayed using the enzyme coupled luciferin/luciferase assay[3,8,9,14]. This reaction produces light (photons) which is directly proportional to the amount of ATP present in the sample[3,11,18]. The light output can be precisely measured in a luminometer or with a CCD camera[16]. Microbial and non-microbial ATP (which usually refers to free ATP not contained within a microorganism) can be differentiated by first measuring the free ATP in a sample followed by the enzymic destruction of the free ATP. Microbial ATP can then be released by lysing the microorganisms present in the sample and can be measured by the luciferin/luciferase assay. However, for most surface hygiene monitoring, total ATP is measured by releasing all ATP from microbial sources and measuring the free and released ATP together.

The use of ATP bioluminescence methods including instant hygiene monitoring based on ATP detection has become very popular in the food and beverage industries[1,6,13,17] including the brewing industry[7,8,9,15,19]. The swab testing methods involve swabbing a surface to pick up residual ATP and microorganisms followed by a step to release microbial ATP. Light produced from an enzyme-coupled assay is then measured in a luminometer and correlated directly to ATP levels in the original sample. This reaction can be accomplished in seconds providing near instantaneous measurements of ATP. If a surface has been properly cleaned and sanitized there should be little or no ATP present (free or contained within microorganisms). However, as residues from the fermentation process and microorganisms are left behind and/or are not removed during cleaning and sanitation, ATP levels are shown to increase. Therefore, the method is theoretically very useful for assaying the effectiveness of cleaning and sanitation.

Silliker Laboratories Group, Inc. investigated five different luminometers and found large differences in their performance[13]. Since it was reported that the minimum concentration of ATP detected by the luminometers varied by more than a hundred-fold, one of the objectives of this study was to determine which is the best current system(s) to use. The main objective, however, was to determine if swab units are effective and reliable at picking up ATP and accurately measuring concentrations in a luminometer.

Davidson, *et al.*[2] reported that an ATP bioluminescence swabbing procedure was superior to standard hygiene swabbing for reproducibility of results, and that standard hygiene swabbing recovered less than 0.1% of viable test bacteria after they were inoculated onto a surface and dried. Since ATP is a relatively stable molecule, it can be recovered from non-viable organisms, and therefore, can theoretically give a more thorough evaluation of cleaning than a plating method relying on viable microorganisms. There have been mixed reports in the literature on the reliability of the swab detection method with some authors concluding that there is variable sensitivity and poor reproducibility[6,12,13] while others have concluded that it is a reliable method[10,15]. Furthermore, it has been shown by Green, *et al.*[4,5] that a number of cleaning and sanitizing agents can interfere with the ATP bioluminescence assay causing either lowered or increased readings depending on the agent and ATP source. It is noted that chlorinated sanitizer and sodium hypochlorite (two of the more commonly used sanitizers in the brewing industry) have little effect on ATP bioluminescence[4,5].

Four different instruments with four different self-contained swab units (designed for use with each of the instruments) were evaluated in the current study. The self-contained swab unit usually contains a pre-moistened sterile swab that is first used to swab a determined area. The swab is then introduced into a buffer/lysing solution to remove the absorbed material from the swab. This solution then enters a chamber where it mixes with the luciferin/luciferase cocktail (usually dehydrated in a tablet form). If ATP is present in the solution, light is produced and is then measured in the luminometer either directly in relative light units (RLU's) or on a logarithmic scale with predetermined "zones of cleanliness".

MATERIALS AND METHODS

Bacteria/Yeast

The following microorganisms were used in the studies:

Pediococcus damnosus – beer spoilage isolate.

Lactobacillus paracasei – beer spoilage isolate.

Saccharomyces carlsbergensis – a lager beer production strain.

Culturing microorganisms

The *Pediococcus damnosus* isolate was grown to stationary phase in Lactobacillus MRS broth (Difco # 0881-17-0). The suspension was then centrifuged and the cells were washed with phosphate buffered saline and resuspended in 10 ml of buffer solution to a concentration of approximately 10^8 cells/ml. Serial dilutions of this suspension were made in sterile, phosphate buffered saline. All serial dilutions were filtered through sterile, 47 mm, 0.45 µm, black grid membrane filters which in turn were plated on Barney Miller Brewing (BMB) agar (US Patent # 4,906,573, Difco # T634-17). The plates were then incubated for 7 days, anaerobically (in a 100 % CO_2 environment) at 28°C. Colony forming units were used to calculate the amount of viable bacteria that were present in the original 10 microliters (µl) of sample used to inoculate swabs as described in the "Swab Detection" section below.

An isolate of lager yeast was grown in 1% yeast extract, 2% peptone and 2% dextrose (YPD) broth to stationary phase. The yeast suspension was serially diluted and filter plated as described above with the exception that YPD agar was used instead of BMB agar. The yeast sample plates were incubated aerobically for 3 days at 28°C. As with the Pediococcus samples, the same serial dilutions were used for swab detection experiments.

The *Lactobacillus paracasei* isolate was cultured on Lactobacillus MRS agar and incubated at 28°C for five days in an anaerobic (100% CO_2) incubator.

A suspension made from yeast grown on YPD agar, *Pediococcus* grown on BMB agar and Lactobacillus grown on *Lactobacillus* MRS agar was prepared by placing several colonies of each into sterile phosphate buffered saline solution followed by thorough mixing. This suspension was then serially diluted in sterile phosphate buffered saline. Aliquots of these dilutions were filtered through sterile, 0.45 µm, black-grid membrane filters, which were then plated on Universal Beer Agar (UBA). Plates were incubated anaerobically in a CO_2 environment at 28°C for 7 days. The surface of a laminar flow hood was washed thoroughly, rinsed several times and finally sterilized with 70% ethanol. Duplicate 10 µl aliquots of the yeast/bacteria suspensions were pipetted onto the sterile surface and allowed to dry (with the hood turned off). Each spot was swabbed according to the directions for each unit.

Bioluminometers

Four different bioluminometers and their self-contained swab kits were evaluated in this study. These were: the IDEXX Lightning® (BioControl, Belleview, WA), the Charm LUM-T (Charm Sciences Inc., Malden, MA), the Biotrace Uni-Lite (Biotrace, Inc. Plainsboro, NJ) and the Celsis-Lumac SystemSURE™ (Becton Dickinson Microbiology Systems, Sparks, MD).

Background ATP Determination

Ten blank swabs were assayed without opening the swabs and running them through the extraction procedure for each instrument to determine the background light measurements. The data from these runs is presented in Table I.

TABLE I. Background ATP readings for blank swabs using four different luminometers and swab sample kits.

Idexx Lightning		Charm LUM-T	Uni-Lite	Celsis-Lumac System Sure
Decade (Log) Reading	Antilog Reading	Relative Light Units (RLU's)	RLU's*	RLU's
1.4	25.1	0	20	10
1.5	31.6	0	29	14
1.4	25.1	0	25	14
1.4	25.1	0	28	10
1.5	31.6	0	26	7
1.6	39.8	0	28	7
1.6	39.8	0	21	13
1.5	39.8	0	22	7
1.9	79.4	0	20	7
1.4	25.1	0	30	13
Ave. 1.5	36.2	Instrument is calibrated to read 0 background	Ave. 25	Ave. 10.2

RLU's = relative light units

ATP standards

ATP frozen standards (supplied at 800 femtomoles (fM) per tube) were thawed and serially diluted for a standard curve for each instrument. Dilutions were made in a sterile, filtered normal saline/Butterfield's buffer solution. A 10 µl aliquot of the standard was pipetted directly onto the tip of each swab. The manufacturer's protocol was followed for determining ATP levels on each individual swab. An ATP standard curve was determined for each instrument by measuring serial dilutions from 800 femtomoles to 0.8 femtomoles of ATP (Fig. 1). In a previous experiment (data not shown) powdered ATP from Sigma Chemicals was dissolved in sterile, ATP free water and diluted to the same levels as the commercial ATP standards. Light measurements were equivalent indicating that the ATP standards were accurate.

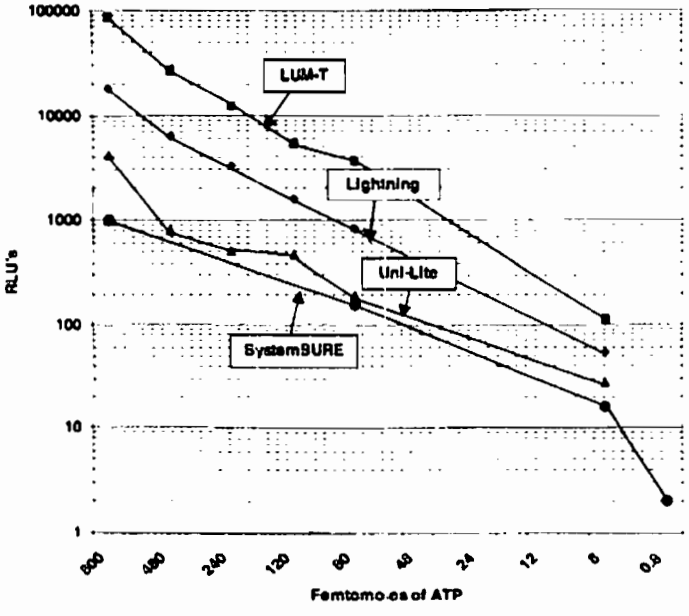

FIG. 1. Measurement of ATP standards using four luminometers and their swab kits.

Swab detection of *Pediococci* and yeast samples

The same serial dilutions of the Pediococcus and yeast cultures described above that were filter plated to determine viable counts were used for the detection of microorganisms on swabs by the luminometers. 10 µl of each serial dilution was pipetted directly onto the center of duplicate, triplicate or quadruplicate swabs recommended for each luminometer. These were then processed as described by the manufacturers and read directly in the luminometers.

RESULTS AND DISCUSSION

Table I shows the results of 10 swab blanks run in each unit. The IDEXX Lightning readings are according to pre-set zones, which provide a decade, reading (which is equivalent to the log of the light output). For comparison purposes, these readings were converted to an antilog reading to put them on a scale equivalent to the relative light units used for the other instruments. The readings for the other three instruments are reported in relative light units (RLU's). It is noted that RLU's vary from instrument to instrument and are not equivalent for comparison. Therefore, none of the units of measurement for the four instruments tested are comparable to each other.

As the data in Table I shows the background readings for the Lightning varied over 300% from 25.1 to 79.4 for the antilog value. The readings for the SystemSURE varied 200% from 7 to 14 RLU's. The Uni-Lite had the

least variation at 50% from 20 to 30 RLU's. Since the LUM-T was set to blank automatically to a reading of 0 RLU's for background, it could not be compared to the other instruments.

The graphs presented in Figure 1 compare the four meters and their swab systems for measuring ATP standards of known concentrations. The Lightning readings were not linear over ATP concentrations of 8 to 800 fM. The corrected average reading for 8 fM was 64 while the corrected average reading for 800 fM was 17,865. Instead of an expected 100-fold increase in the reading, the reading increased 279-fold. It is noted that the lowest standard of 8 fM of ATP gave a reading significantly higher than the background average in Table I. The reading was approximately three times higher than background, but all four instruments tested claimed that the lower limit of accurate measurement was 0.2-0.4 fM of ATP. Based on the background reading the lower limit of detection for the Lightning and its swab system would be estimated to be 2.5 fM ATP. It is also noted that the reading for 800 fM ATP was 20 times greater (instead of the expected 10 times) than the reading at 80 fM ATP.

Swabs with the LUM-T also did not provide readings that were linear over the ATP concentrations tested. The replicate sample readings were very erratic using the swab system. For example: The average reading for a sample with 8 fM ATP pipetted directly onto the tip of the swab was 115 RLU's, but two of the triplicate samples actually gave readings of 0 while the third had a reading of 346. This would suggest that the reliable lower level of detection for this instrument using a swab system might actually be greater than 8 fM ATP.

The Uni-Lite gave an average reading at 8 fM ATP that was only twice that of the 0.8 fM ATP control. Again this would indicate that with the swab system, the minimum detection limit should be around 4 fM which is 10 times higher than what the manufacturer claims. The reading at 800 fM ATP was approximately twenty times greater (instead of the expected ten times) than the reading at 80 fM ATP demonstrating that there was not good linearity in readings over a range of ATP concentrations.

The SystemSURE (which was tested later than the other three) was tested over a larger range of ATP concentrations. The reading at 0.8 fM was not significantly different from background (compared to the readings in Table I, which varied from 7-14 RLU's). The reading at 8 fM was 8 times greater than the reading at 0.8 fM ATP; the reading at 80 fM was 9 times greater than the reading at 8 fM ATP and the reading at 800 fM was 6.8 times greater than the reading at 80 fM. For perfect linearity the readings should have been exactly 10 fold increases, but none the less the SystemSURE gave the most linear results of the 4 instruments tested for using known quantities of ATP on the swabs.

The SystemSURE also had a CIP rinse water assay kit available for performing liquid assays of ATP standards directly in tubes without swabs. Figure 2 presents a graph of the data where ATP standards were serially diluted and read in test tubes without swabs. In this case the SystemSURE was shown to be sensitive down to 0.4 fM of ATP (with one RLU = 0.4 fM of ATP) which corresponded to the manufacturer's claim. As the graph indicates, the data was linear over a range of dilutions. Since ATP standards in liquid samples gave more linear readings using the bioluminescence assay than did swab tube samples, it might indicate that the swab interferes with the assay or the light reading. Inconsistent readings and poor linearity with swab samples appear to be due to the nature of the swab method and not the instrument.

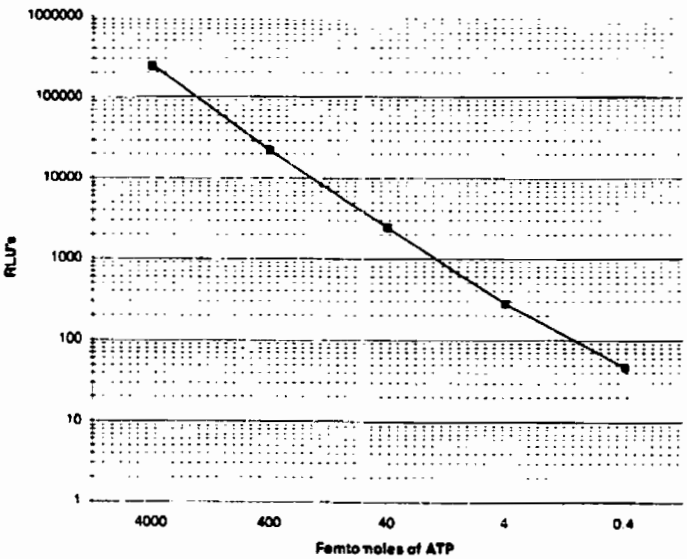

FIG. 2. Detecting ATP standards using the SystemSURE CIP Rinse Water Kit.

The graphs in Figure 3 compare the four instruments and their swab systems for detecting various concentrations of a *Pediococcus sp.* that were pipetted directly onto the tips of the swabs. If a meter and its swab detection kit is accurate in detecting bacteria at different concentrations (i.e. linear), it should provide an ATP reading ten times greater for 36,600 colony forming units (CFU's) per 10 µl than that for 3660 CFU's per 10 µl. The Lightning gave a decade scale reading of 2.13 (antilog minus background = 101) for 3660 CFU's and a decade scale reading of 2.5 (antilog minus background = 316) for 36,600 CFU's (i.e. only 3.1 times greater). A decade reading of 3.13 would have been expected if measurements were linear. The LUM-T provided readings in the opposite direction. 3660 CFU's gave an average reading of 544 RLU's (with a variance from 383 to 741 RLU's for triplicate samples) while 36,600 CFU's gave an average reading of 11,112 RLU's (i.e. 20 times greater). Uni-Lite and its swab kit performed very poorly with 36,600 CFU's giving a background corrected reading of 61 RLU's while 3660 CFU's gave a reading of 21 RLU's.

Again the SystemSURE was tested at a different time than the other instruments, and the *Pediococcus* concentrations did not range as high as in the other tests. 30 CFU's gave a reading lower than the average background measurement, 300 CFU's gave an average reading of 10 RLU's greater than background and 3000 CFU's gave an average reading 100 RLU's greater than background. This represented very good linearity for two readings, but the meter was not available to repeat experiments at higher concentrations. It is also noted that at the 3000 CFU's concentration that the actual readings for triplicate samples showed wide variation from 70 to 187 RLU's.

The SystemSURE and its swab kit showed the best sensitivity for being able to detect the test Pediococcus. It detected 300CFU's while none of the others detected this organism at 366 CFU's. According to the all of the manufacturers' specifications, the instruments should be able to detect 100-1000 bacteria/sample. High variability of replicate sample readings was obtained with all of the concentrations of *Pediococcus* tested with all of the instruments. This may be due to the inability of the lysing agent to extract the ATP from the cells reliably or to unacceptable light transmission through the swab.

Figure 4 compares graphs of the data using the four systems to detect different concentrations of yeast that were pipetted directly onto the tips of the sample swabs.

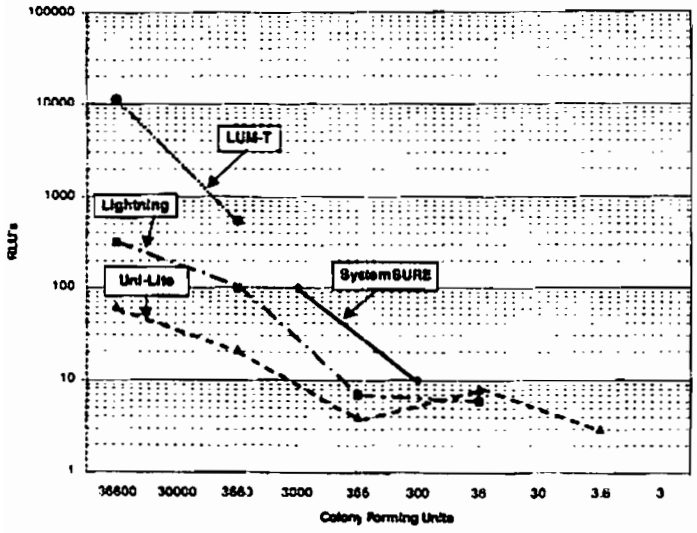

FIG. 3. *Pediococcus* measuring using four different ATP bioluminometers and their swab systems.

The data shows that the Lightning system was fairly linear at detecting 38 and 383 yeasts, but as the counts were increased to 3830 and 38300 the readings dropped to half of that expected. The variation in replicate readings was improved over that seen with bacteria indicating that ATP was probably being extracted more efficiently from yeast. The LUM-T system gave more linear results based on the average RLU's of triplicate samples, but showed higher variation between individual samples. For example with a count of 38 yeast cells the readings varied form 216 to 1219 RLU's and with 3830 yeast cells the reading varied from 42,577 to 85,560 RLU's. The Uni-Lite system did not produce a reading significantly above background for 38 yeast cells but did detect 383 yeast cells. 3830 yeast cells then gave an average reading twelve times that of the 383 sample (854 RLU's compared to 69 RLU's), but the 38300 yeast sample gave a reading only 5.8 times the 3830 yeast cells measurement (4954 RLU's vs. 854 RLU's). As with the LUM-T system the individual readings for the triplicate samples using the Uni-Lite system displayed a very wide variance (e.g. for 383 yeast cells readings varied from 64 to 123 RLU's and with 38300 yeast cells readings varied from 1692 to 8187 RLU's). The SystemSURE system was less sensitive than the other instruments tested for detecting yeast. 210 yeast cells gave a reading only slightly higher than background. The average readings for the increasing yeast concentrations were fairly linear. Subtracting out the background the readings were 4 RLU's for 210 yeast, 23 RLU's for 2210 yeast, 263 RLU's for 22100 yeast and 1962 for 221000 yeast. However, as with two of the other instruments there was wide variation in the individual reading for the triplicate samples.

It is noted that the SystemSURE provided a reading of 23 RLU's for 2,100 colony forming units of yeast (Fig. 4) while it gave a reading of 100 RLU's for 3,000 colony forming units of bacteria (Fig. 3). Since yeast typically contain 50-100 times more ATP than bacteria, it is suspected that the lysing agent used in the SystemSURE swab kits does not efficiently extract ATP from yeast. The other three instruments gave the expected proportionally higher RLU's for yeast cells compared to bacterial cells probably indicating that they more effectively extracted the ATP from the test yeast.

However, in general for detecting yeasts all of the instruments' readings fell below the expected readings as the yeast counts increased. Also, all of the instruments showed wide variability in replicate samples. Both of these factors might suggest that there is incomplete extraction of ATP from the cells and/or the swabs may be inconsistently interfering with light detection.

Figure 5 presents the data from the last experiment where the four instruments and their swab units were evaluated for recovering a dried mixture of bacteria and yeast from a smooth surface. All of the meters and their swab systems gave erratic and non-linear results for detecting increasing concentrations of microorganisms dried on a surface. For example, the Lightning system gave an adjusted reading (antilog) of 861 for 3300 microorganisms and a reading of only 3571 for 330,000 microorganisms. This is less than a 10-fold increase in the reading for a 100-fold increase in microorganisms. The LUM-T system gave duplicate readings of 0 and 485 RLU's for duplicate swabs of 3300 microorganisms and

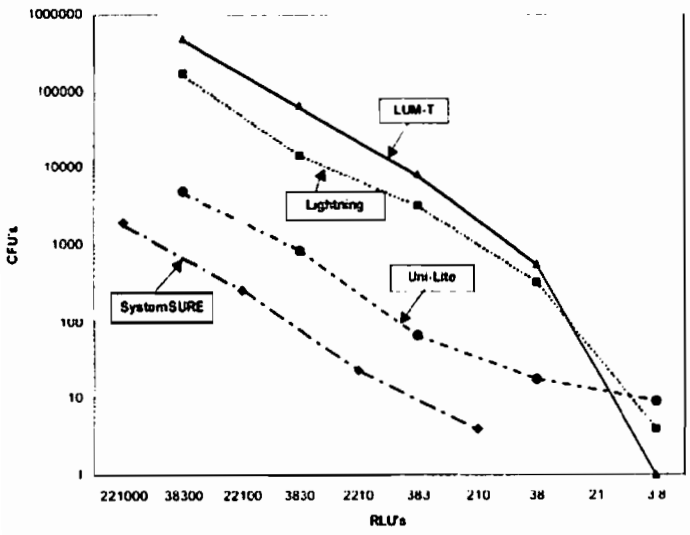

FIG. 4. Yeast measurement using four ATP Bioluminometers and their swab units.

duplicate readings of 0 and 0 RLU's for 330,000 microorganisms. The Uni-Lite system gave an average corrected reading of 496 RLU's for 3300 microorganisms and a reading of 1170 RLU's for 330,000 microorganisms. The SystemSURE gave an average corrected reading of 305 RLU's for 5,000 microorganisms and an average reading of 5209 RLU's (ranging from 382 to 9247 for triplicate samples) for 5,000,000 microorganisms.

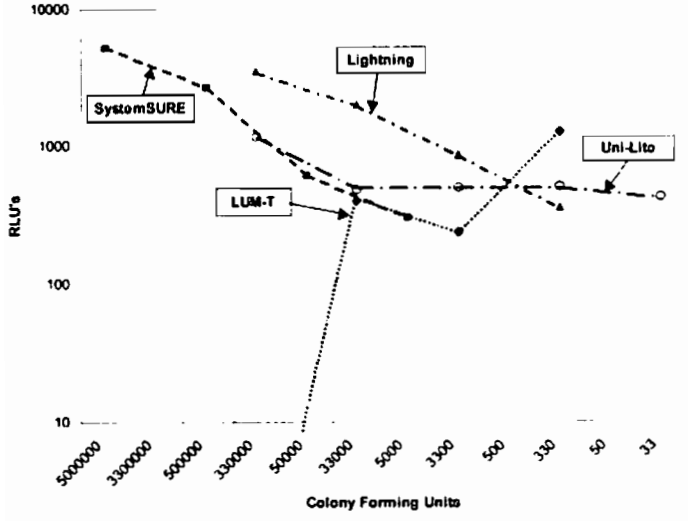

FIG. 5. Detection of bacterial/yeast suspension dried on a surface using the swab detection method.

CONCLUSIONS

All four ATP bioluminometers and their swab systems tested in this study were inconsistent at detecting ATP. None of the meters tested with their swab units gave linear results for detecting increasing known concentrations of ATP standards pipetted directly onto the tips of swabs. SystemSURE gave the closest to linear results when comparing average readings of replicate samples. However, all of the meters gave inconsistent readings for replicate samples. None of the systems was consistent at detecting microbial ATP from bacteria or yeast over a range of increasing cell densities pipetted directly onto the tips of the swabs. None gave linear results for increasing concentrations of bacteria. SystemSURE detected bacteria at the lowest level at 300 *Pediococci* per swab while the other meters required around 3000 bacteria per swab to produce a reading significantly greater than background. All of the meters gave inconsistent readings for replicate samples containing the same concentration of bacteria. The sensitivity for detecting yeast for the four instruments was different than that obtained with bacteria. SystemSURE required over 200 yeast per swab for detection while the Lightning and LUM-T systems required 38 (or less) yeast per swab. The SystemSURE did not give proportionally higher relative light units for yeast detection (on a per cell basis) compared to bacteria possibly indicating that the lysing agent was ineffective with yeast cells. As with bacterial and ATP standards detection, all of the instruments and swab systems gave inconsistent readings for replicate yeast samples.

All of the meters and swab samples performed very poorly and inconsistently at detecting microorganisms dried on a hard surface. None gave linear results for increasing concentrations of microorganisms. When levels of microorganisms were increased one thousand fold, none of the meter readings increased as much as 20-fold.

The main conclusion from this study is that of the meter and swab systems tested none gave reliable results for consistently detecting microorganisms. Since one of the meters was tested with a tube assay for liquid samples and showed good linearity and reproducibility, it is suggested that the swab method itself is unreliable. A possible explanation for inconsistent measurements for ATP standards could be: 1) that ATP adsorbs to the swab and is not consistently released into the buffer for the enzyme assay or 2) that the swab itself inconsistently blocks or diffracts light being produced in the assay. Inconsistent detection of microorganisms pipetted directly onto the tips of the swabs could indicate that the systems aren't able to efficiently release ATP from microbial cells as well as the two other possibilities listed above. The systems performed the worst at actually removing and detecting microorganisms on a surface using the swab systems. In additions to the other factors mentioned above, the swabs may not adequately pick up microorganisms.

All of the instruments proved unreliable at detecting microorganisms or free ATP using swab systems, and none of the instruments appeared to have any major advantage over the others.

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